Physicochemical Characterization of a Novel Strawberry Confection for Delivery of Fruit Bioactives to Human Oral Mucosa

THESIS

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By

Erica Lauren Fisher

Graduate Program in Food Science and Technology

The Ohio State University

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Master's Examination Committee:

Yael Vodovotz, Advisor

Steven J Schwartz

Christopher M Weghorst
Abstract

Oral diseases are a common health concern in the United States, and studies have revealed that poor oral health may lead to increased risk of certain chronic diseases. Strawberry phytochemicals, such as ellagitannins, anthocyanins, flavonols, and catechins, are known to elicit health benefits by anti-inflammatory and anti-oxidant properties that can improve oral health. A unique starch-based confection containing freeze-dried strawberries was developed to provide sustained, targeted release of phytochemicals to the oral mucosa to promote oral health in a human clinical trial. The objective of this study was to compare physicochemical properties of the confection with in vitro dissolution studies to evaluate the facility of the confection to sustain phytochemical release in the oral cavity in a phase I/II clinical trial.

Water distribution was assessed with thermogravimetric analysis. Texture profile analysis and small oscillatory amplitude testing were used to characterize the behavior of the gel structure under deformation as it related to oral processing. In vitro dissolution kinetics in artificial saliva was evaluated for release of total phenolics and total monomeric anthocyanins. Stability of the freeze-dried strawberry confectionery was assessed during a fourteen-day storage period.

The moisture content was determined to be approximately 20% and decreased during storage, which should be taken into account when manufacturing the confections.
for a clinical trial as changes in moisture content and water dynamics will impact the
shelf stability. It was also determined that hardness, gumminess, and chewiness varied
significantly with storage. Rheological characterization of the confectionery showed that
the behavior of the microstructure of the food matrix under deformation was frequency-
and shear-dependent. Throughout oscillatory frequency testing, the confectionery
exhibited a higher elastic modulus than viscous modulus, indicating a more solid-like
behavior upon deformation. Similarly, the viscosity of the confection decreased in a
Newtonian-like manner until reaching the time constant shear value, at which
pseudoplastic behavior was observed in the range of frequencies typically observed
during oral manipulation of semisolid foods. Temperature changes between room
temperature and body temperature showed minimal effects on the elastic and viscous
moduli. *In vitro* dissolution testing revealed that after six hours of testing, 83% total
phenolics and 86% monomeric anthocyanins were released.

A phase I/II pilot clinical trial was conducted to evaluate the efficacy of
strawberry bioactives delivered by the novel confectionery to prevent oral disease in
smoking and non-smoking men and women. Participant compliance to diet, smoking, and
confectionery consumption was evaluated. It was found that the optimized confectionery
formulations were well-tolerated and subject compliance was good. The strawberry
confectionery showed great potential as a novel vehicle by which fruit bioactives may be
delivered to oral tissue. Further analysis of biological samples is needed to determine the
tissue deposition and subsequent gene modulation of the novel functional confectionery.
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Vita

June 2006 ...................................................... Graduate, William Mason High School

March 2010 ................................................... Bachelor of Science in Food Science and Technology, The Ohio State University

March 2010- September 2011 ....................... Graduate Research Associate,

Department of Food Science and Technology,

The Ohio State University

Publications


Fields of Study

Major Field: Food Science and Nutrition
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Introduction

1.1 Context of Project

Phytochemicals found in strawberries have been shown to provide numerous health benefits. Specifically, ellagitannins, anthocyanins, flavonols, and catechins, are naturally-occurring compounds found within strawberries and are known to confer health benefits by anti-inflammatory, anti-oxidant, and chemopreventive properties (Hannum, 2004; Seeram, 2007). As such, strawberry phytochemicals may elicit protective action against oral maladies when delivered to mucosal tissue in therapeutically effective doses.

Confections are a large snack food market within the United States, and as such may be a novel functional food product. Incorporating whole strawberries into the confectionery formulation and provide individuals with the naturally-occurring bioactive compounds to promote oral health and prevent disease. Starch-based confections are amorphous solids that offer many advantages over traditional crystalline pharmaceutical forms, including increased solubility, recovery upon compression, and can be readily formulated from low-cost biopolymers (Lian, 2001). By manipulating a food formulation to create an amorphous semisolid excipient that delivers phytochemicals in a therapeutically effective dose, the health benefits of naturally-occurring foods can be targeted to specific tissue. While amorphous solids offer advantages over crystalline forms, they are typically not as
stable and require characterization of changes during storage. A thorough investigation of physicochemical interactions is necessary to modulate release of bioactives in a specific tissue site and ensure consistent functionality. The food matrix must be durable enough to maintain its structure long enough to delivery bioactive compounds to the target tissue, yet still offer desirable taste, texture, and convenience. Shelf-stability, reproducibility, and desirable functionality are determined by physical properties of food products and further necessitate an understanding of physicochemical interactions.

1.2 Statement of Problem

Convenient deposition of health-promoting fruit bioactives that have known health benefits may provide a novel treatment and prevention method for oral maladies. It was hypothesized that the physical properties of a starch-based confectionery excipient containing freeze-dried strawberries would attenuate sustained release of bioactives to the oral mucosa. To investigate the hypothesis, the following research aims were ascertained:

Aim 1: Develop an optimized freeze-dried strawberry confection using physicochemical analyses including: thermogravimetric analysis, large and small deformation testing, and dissolution testing.

- Manufacture shelf-stable confectionery dosage form containing at least 45% freeze-dried strawberry powder
- Physical characterization of the strawberry confections with large and small deformation testing and thermogravimetric analysis.
- Dissolution behavior of the confection analyzed in vitro in biorelevant medium
• Time-dependent changes will be evaluated by the same physical characterization methods to assess deleterious changes

Aim 2: A phase I/II pilot clinical trial will be conducted to assess subject compliance with self-reported daily consumption records and to establish minimal toxicity in daily doses of strawberry confections

• Recruitment and accrual of smoking and non-smoking men and women for participation in a six-week clinical trial

• Assess subject compliance with consuming four doses of study confections for seven days of treatment period

• Evaluate self-reported confection dissolution rates from participants collected throughout seven day treatment period
Chapter 2: Literature Review

1.3 Functional Starch Confectionery

1.3.1 Confectionery Products

Recent consumer trends have shifted toward procuring food products with a wide spectrum of health benefits. Consumers today want healthful foods that provide phytonutrients to promote good health and well-being without sacrificing taste, texture, or convenience. As a result, food product development has shifted toward creating innovative products that meet the demand for natural, health-promoting compounds in food forms that are familiar to consumers. Top confectionery producers recognize that enriched gummy confections such as chocolates and candies with added vitamin C, fiber, and fruit juices are a new major trend amongst American candy makers (Burroughs, 2010) and may provide a unique manner by which health-promoting compounds can be consumed. Globally, manufacturers of medicated confectionery, fortified and functional gum, and fortified and functional chocolate saw sales of over $11 billion (Maloughney, 2011). Functional foods provide a means by which consumers can supplement their diets with foods that are similar to conventional products but contain natural, healthy bioactives from other whole-food sources such as fruits, vegetables, legumes, fish, and
nuts (Shahidi, 2004). Health-conscious individuals seek food products that can deliver health-promoting compounds without sacrificing gustatory features and can be eaten as part of a usual diet. Consumption of fruits is part of maintaining a healthy lifestyle, but it was estimated that one in ten American adults do not consume the recommended amount of fruits and vegetables (Kimmons, Gillespie, Seymour, Serdula & Blanck, 2009). Therefore, confectionery forms can provide a convenient way in which Americans may consume health-promoting compounds from nutritious foods that are incorporated into the functional food formulation. Confectionery products are food formulations characterized by aqueous dispersions of sugar syrups and are available in a broad variety of forms including caramels, marshmallows, gums, jellies and gummies, and hard candies (Figure 1). Soft jelly or gummy confectionery products are characterized by a soft and chewy texture typically conferred by a gelatin or pectin-based gel (Burey, Bhandari, Rutgers, Halley & Torley, 2009; Warnecke, 1991). Flavorings and colors are readily added to soft confectionery products (Burey & others, 2009; Warnecke, 1991).
Figure 1. Variety of confectionery forms including fruit gummies, cotton candy, caramels, rock candy, marshmallows, and hard lollipops.

1.3.2 Starch-based Confections

Gelatin, the traditional gelling agent used in gummy confectionery formulations derived from animal protein, is losing favor due to cultural, religious, and financial concerns (Lennox, 2002). To continue to meet the demand for soft, gummy confections, starch-set confectionery products are a unique alternative to gelatin-based confections (Karim & Bhat, 2008). Such starch-based confectionery gels are formed by “thin boiling” or “acid-thinned” starches that are formed by adding a small amount of acid to a starch suspension and heating at a temperature below the gelatinization temperature to yield starch hydrolysis (Burey & others, 2009. The resulting hydrolyzed starch is readily soluble in boiling water and disintegrates when cooked to give a lower hot paste viscosity and higher gel viscosity than non-acid modified starches, yet retains its granular structure (Wang & Wang, 2001; Wang, Truong & Wang, 2003). Gels formed by acid-thinned
starches have desirable properties for soft confection applications, such as greater acid and heat stability, shorter gelation time than gelatin gummies, and relatively low hot viscosities and can therefore be agitated and pumped with minimum energy expenditure (Karim & Bhat, 2008; Wang & Wang, 2001). Acid-thinning can decrease granule amylose content as amylose is more easily cleaved by acid hydrolysis than amyllopectin. This causes a decrease in crystallinity of the starch, and yields a more amorphous material, which allows starch processing for food applications to be easier (Damodaran, Parkin & Fennema, 2008; Wang & Wang, 2001). The gelatinization temperature and the breadth of the gelatinization endotherm have been shown to increase on acid hydrolysis of starch. Acid modification has been found to increase solubility and gel strength and decrease viscosity of starches. The viscoelastic properties of starches are also affected by acid hydrolysis (Wang & Wang, 2001).

Carbohydrates are the most widely-consumed energy source in the Western diet (Austin, Ogden, & Hill, 2011). The Centers for Disease Control estimated that the mean percentage of daily calories from carbohydrates has increased from about 40% in 1971 to nearly 50% in 2000 (Austin, Ogden & Hill, 2011). Starch is one of the most abundant carbohydrates in nature and can be obtained from plant sources such as maize, wheat, potato, and rice (Burey, & others, 2009). In the food industry, starches are used for their water binding ability, adding viscosity, providing bulk and stabilizing components of the food matrix (Damodaran & others, 2008). Physical properties of foods imparted by starch are based on the chemical composition of the starch. Dynamic chemical interactions such
as ionic bonding, hydrogen bonding, and hydrophobic interactions between neighboring starch components as well as lipids and proteins within a food matrix allow for a variety of physicochemical food properties (Chinachoti, 1995).

The structural properties of amylose and amylopectin in the starch granules (e.g. average molecular weight, frequency of branching in amylopectin, naturally occurring level of phosphorylation) depend on plant source (Burey & others, 2009; Chinachoti, 1995). Starch is arranged in semi-crystalline granules within plant cells. The granule consists of two different polysaccharides, amylose and amylopectin. Amylose is a linear glucose chain formed by α1,4-glucosidic bonds with a degree of polymerization ranging between 250 – 1000, whereas amylopectin is a branched glucose chain comprised of α1,4-glucosidic bonds with α1,6-glucosidic bonds at branches that allows for a degree of polymerization greater than 6000 and up to 3.0 million (Chinachoti, 1995). When using starch in a food system, it is necessary to understand the functionality of the specific starch to ensure minimal variability in the manufactured product. As the market demands for healthier, more “natural” food products grow, the uses of starch become more varied. For application within a confectionery gel system, physical properties such as pasting viscosity, gelatinization temperature and rate of retrogradation are also dependent on the plant source of the starch (Burey & others, 2009). Understanding these physicochemical properties of starch in such a gel is important for ensuring desirable sensory attributes in the final product.

1.3.3 Starch Functionality
Gelatinization of starch is a physicochemical property requiring attention in the confectionery gel application. Starch gelatinization occurs as the tightly organized crystalline structure of the starch granule is lost upon application of heat in an aqueous environment (Chinachoti, 1995). This phenomenon occurs when starch granules are heated above a specific gelatinization temperature (typically between 56 – 68 °C for native starch); the granule swells and leaches amylose, and upon cooling forms a rubbery gel through hydrogen bonding between water and free hydroxyl groups on the amylose chains as presented in Figure 2. (Chinachoti & Chatakanonda, 2010). Gelatinization yields a thermally-irreversible, viscous gel that provides a soft, springy texture or a brittle, easily fractured texture depending upon the starch structure and slurry components. Other food components in the gel, such as sugars, salts, lipids, surfactants, polyphenolics, and the amount of water, may alter the gelatinization of the starch (Zhu, Cai, Sun, & Corke, 2008; Mohamed & Rayas-Duarte, 2003; Chinachoti, 1995). Starch requires approximately 30% moisture to fully gelatinize (Burey & others, 2009). (Gunaratne, Ranaweera, & Corke, 2007) found that sucrose and glucose can increase both the gelatinization temperature and gel hardness in wheat starch gels, but the effect is more pronounced with sucrose. Upon formation of a gel, the components of the disrupted starch granule can re-associate in a process known as retrogradation (Abd Karim, Norziah, & Seow, 2000).

Shortly after gelatinization, amylose crystallizes and a phase separation occurs between the amylose and aqueous solution (Abd Karim, Norziah, & Seow, 2000). After prolonged
storage, amylopectin polymers can reorder as outer branches crystallize. Starch retrogradation can impart undesirable sensory features to a food product, specifically coarseness, firming, or precipitation of starch components, and must be considered when developing a new food product. In addition to the chemical composition of the starch granule, it has been shown that temperature fluctuations (such as freezing and thawing), moisture content and relative humidity fluctuations can alter the rate of starch gel retrogradation (White, Abbas & Johnson, 1989; Sandhu & Singh, 2007). Macroscopic instrumental techniques can be used to identify changes in a food matrix due to gelatinization and retrogradation; specifically, rheological analysis, sensory evaluation, DSC, or thermogravimetric analysis.

Figure 2. Native starch granule (A) gelatinizes (B) and then retrogrades (C) (Wageningen University)

1.3.4 Water Dynamics in Confections

Water is added to confectionery products to dissolve the gelling agent and sweeteners, colors, and flavors. Shelf life, texture, and ingredient stability are all directly related to the water dynamics within confectionery products and most other food systems (Roos, 1995). The water content of gummy or jelly-like confections is typically between 8 –
22%, whereas the water content of hard candy is only about 2-5% (Ergun, Lietha & Hartel, 2010). Through dipole-dipole interactions, ionic bonding, hydrogen bonding, and van Der Waals forces, water molecules interact with solutes in the confectionery matrix and can be categorized as freezable, unbound water or non-freezable, bound water (Damodaran & others, 2008; Ergun, Lietha & Hartel, 2010). Dynamics of water in food and polymer systems are typically described by measurements of water activity, water mobility, and moisture migration. Water will promote gel formation by acting as a plasticizer for the starch (Whistler, BeMiller & Paschall, 1984; Ryan & Brewer, 2005), and can also affect the glass transition temperature of the starch (Damodaran & others, 2008). Plasticized amorphous regions of the starch granule undergo a phase transition from glassy to rubbery upon heating above the gelatinization temperature (Damodaran & others, 2008). Additionally, the migration of water between the confection and the ambient environment can impact physicochemical attributes and potentially lead to hardening (moisture loss) or softening (moisture gain) (Roos, 1995). Moisture migrates from the food matrix to the environment if the water activity is greater than the relative humidity (Ergun, Lietha & Hartel, 2010). As such, it is critical to maintain ambient relative humidity less than the water activity of the confections to ensure the product maintains good quality.

1.3.5 Calorimetric Testing

To determine the energy content of the freeze-dried strawberry and placebo confectionery products, Bomb calorimetry was employed. The basic theory behind bomb calorimetry involves incinerating a sample of known mass in chamber flushed with oxygen. The
energy loss from the sample upon ignition heats a known volume of water surrounding the chamber (Parr Instrument Company, 2004). Work done on the system is zero so that the increase in temperature of the water bath is correlated to the energy of the sample (Parr Instrument Company, 2004).

1.3.6 Physical Properties of Confectionery Products

The viscoelastic nature of starch confectionery products provides a novel functional food system by which fruit inclusions or extracts can be consumed for nutritional and health benefits while still maintaining the pleasurable taste and texture of traditional gelled confectionery products. The widely accepted definition of a gel is a three-dimensional network with a solid substance finely dispersed or dissolved in a continuous liquid phase that will exhibit solid-like behavior upon deformation (Burey & others, 2009). Commercial food gels are a mixture of biopolymers that impart texture, flavor release, appearance, and mouthfeel unlike other food products (Burey & others, 2009; Grant, 2005). Examples of common food gels include: cheese, tofu, imitation crab (surimi), pudding, and soft confections. A confectionery gel consists of gelling components such as starch, gelatin, or pectin, sugars or sweeteners and may contain acids, flavorings, colorings, and nutraceutical additions (Burey & others, 2009; Grant, 2005). Confectionery products can be categorized based on their molecular mobility as crystalline or amorphous solids, with crystalline molecular organization being the most stable (Ergun, Lietha & Hartel, 2010). Confections with amorphous molecular arrangements can further be described as rubbery or glassy, depending on the temperature at which the molecular composition of the food exhibits a glass transition (Ergun, Lietha...
Physicochemical interactions between components of the confection will dictate the taste, texture, and stability of the product. For example, firmness of a starch gel is expected to increase over time as a consequence of starch changing from an amorphous to a more orderly and crystalline state (Whistler, BeMiller & Paschall, 1984). Addition of soy protein isolate to confectionery starch gels has been shown to decrease the hardness of confections over time in a dose-dependent manner (Siegwein, Vodovotz, & Fisher, 2011). Release of bioactives from starch confectionery gels can be manipulated or enhanced by physicochemical parameters related to the gel microstructure.

1.3.7 Optimization of Physical Properties

In the development of a food or pharmaceutical product, it is necessary to assess the limitations of the formulation during and after processing. For example, acid-thinned starches have shown slower retrogradation rates than native starch often due to amylopectin recrystallization (Damodaran & others, 2008) and retrogradation affects quality, acceptability, and shelf life of starch gelled products (Burey & others, 2009). It is therefore reasonable to expect physicochemical changes within the confectionery matrix upon storage that may alter bioactive release kinetics. The interaction of water, carbohydrates, proteins, and other molecules contribute to the physical properties of the gel. Water will promote gel formation by acting as a plasticizer for the starch (Whistler,
BeMiller & Paschall, 1984; Ryan & Brewer, 2005), and can also affect the glass transition temperature of the starch (Burey & others, 2009). Plasticized amorphous regions of the starch granule undergo a phase transition from glassy to rubbery upon heating above the gelatinization temperature (Damodaran, Parkin & Fennema, 2008). Available water is also affected by the presence of proteins. The addition of polymeric compounds, such as starch and protein, to a sugar solution tends to increase the gelatinization temperature, slowing crystallization of amorphous sugars due to decrease free volume, molecular mobility, and diffusivity (Gunaratne, Ranaweera, & Corke, 2007; Sandhu & Singh, 2007). It was found that polyphenolic compounds and phytochemical extracts can alter gelatinization properties of native wheat starch (Zhu & others, 2008; Zhu, Cai, Sun & Corke, 2009). Using calorimetric, rheological, and mechanical means to characterize the soft gelled confections is useful for understanding the physical properties of the gel, and in turn applying that knowledge to making a more therapeutically effective bioactive dosage form.

Characterization of the gel excipient microstructure can be accomplished by texture analysis, rheological analysis, microscopy, sensory evaluation, and dissolution testing (Burey & others, 2009; Azarmi, Roa & Löbenberg, 2007). When starch or starch-containing food is eaten, salivary α-amylase readily hydrolyzes the gelatinized starch to dextrins, maltoose, and maltose (Vesterinen, Myllärinen, Forssell, Söderling & Autio, 2002). Small-strain oscillation showed a strong correlation between the storage modulus, a measure of a gel’s rigidity, and the extent of in vitro starch hydrolysis in a high-
amylose maize starch gel (Vesterinen & others, 2002). That is, a more rigid gel will likely retain its structure throughout oral processing longer than a less rigid, more elastic gel. Furthermore, oral manipulation of semi-solid foods alters the physicochemical properties of the food by dilution with saliva, shear, heating to body temperature, and chemical digestion (Prinz, Janssen & de Wijk, 2007; Janssen, Terpstra, de Wijk & Prinz, 2007). Characterization of the physical properties of a semisolid food system can be predictive of its performance within the oral cavity in terms of subject compliance and acceptance, and of response to oral processing and subsequent bioactive release.

1.3.8 Thermogravimetric Analysis

Thermogravimetric Analysis is used to understand the physicochemical processes that occur in a system by measuring the mass of a substance as a function of temperature (Dollimore, 1990). The sample could be heated or cooled at a defined rate and may have a slower rate at temperature regions of rapid mass change (Haines, 2000). As a result, physicochemical analysis of a solid substance can be determined either by mass loss or mass gain. For example, adsorption, absorption, and oxidation can be determined by a mass gain, whereas dehydration, desorption, decomposition, reduction, and vaporization can be realized upon mass loss (Dollimore, 1990). The atmosphere within the gravimetric analyzer apparatus may be changed, more specifically a vacuum may be applied or an inert gas such as nitrogen can be used (Dollimore, 1990). Obtaining the derivative of the mass loss curve can provide information about the rate of mass loss and corresponding functional properties of the solid system (Haines, 2000). TGA can be used to quantitatively assess the amount of a substance in a mixture or the purity of a substance.
(Dollimore, 1990; Haines, 2000). Thermogravimetric analysis (TGA) was used to identify water distribution shown as mass loss upon heating confection samples.

1.3.9 Rheological Characterization

To further characterize the physical properties of the gel, rheological behavior can be assessed. The science of rheology is concerned with microstructural flow and deformation of a substance in response to applied forces. More specifically, rheology defines relationships between the stress acting on a substance and the subsequent strain (deformation) and/or flow that occurs as either Newtonian or non-Newtonian (Rao, 2007; Tabilo-Munizaga & Barbosa-Cánovas, 2004). Stress (r) is a measurement of force per unit of surface area and is expressed in units of Pascals (Pa). Strain is a dimensionless quantity of relative deformation and the direction of applied stress with respect to the material surface will determine the type of strain. When the applied stress is normal to the sample surface, a normal strain (e) occurs (compressive stress) (Tabilo-Munizaga & Barbosa-Cánovas, 2004). In dynamic oscillatory rheometry a specimen is subjected to a small amplitude strain where the strain is applied at a given frequency ((Rao, 2007, Tabilo-Munizaga & Barbosa-Cánovas, 2004). Typically, the response to the strain is measured in terms of the solid-like properties, or elastic modulus (G’), or the liquid-like properties, viscous modulus (G’’). The phase angle between G’ and G’’ is known as tangent δ and in Newtonian fluids is equal to 1. When tanδ < 1, the substance is considered a viscoelastic solid, and is typically the case with semisolid gelled food systems (Rao, 2007). Most gelled food products have both elastic and viscous properties, and are therefore considered viscoelastic and it can be expected that tanδ will remain less
than one when the deformation force is within the linear viscoelastic range (Abd Karim, Norziah, & Seow, 2000).

Flow analysis can also be used to characterize the rheological behavior of a substance. The resistance to flow of the material is measured in response to shear and typically defined as viscosity (η) or stress (Pa) (Rao, 2007). The relationship between viscosity and shear rate categorizes fluids or semisolids as either Newtonian, in which the viscosity responds to shear in a linear manner, or non-Newtonian, in which viscosity changes in response to shear but not in a completely linear manner (Rao, 2007). Viscoelastic materials typically exhibit a linear response in both the viscous and elastic moduli. However, changes in the polymer organizing with increasing shear can lead to time-dependent changes in the response to shear. It has been observed that semisolid biopolymer gels can exhibit pseudoplastic or shear-thinning behavior in which a quick decrease in viscosity is observed (Rao, 2007). Other changes of the gel microstructure can be realized in terms of temperature, especially in the context of a fruit bioactive dosage form. Intraoral temperature changes and lubrication by saliva will likely alter the response of the gel to oral processing (Prinz, Janssen, & de Wijk, 2007). Lastly, it is important to understand that rheological behavior of starch systems will change as retrogradation occurs. Therefore, monitoring the viscous and elastic components over time is necessary (Abd Karim, Norziah, Seow, 2000).
Rheological characterization of confectionery products can provide a profile of the gel microstructure in terms of gel strength and viscosity, as well as establish parameters that can be related to sensory, dissolution and textural analyses (Burey & others, 2009)). Creamy mouthfeel and after-feel of custards were best predicted using a combination of either G’ or G” values at critical stresses. It was found that critical stress and strain values were highly correlated (r=0.9) and also related significantly with creaminess (Janssen & others, 2007). According to Jianshe (2009), viscosity measured at a shear rate of 10s⁻¹ gave the best correlation to the viscosity perception from sensory evaluations. However, the exact magnitude shear rates within the mouth remains unknown but estimates range from 0.1 or 10 to 1000/s (Jianshe, 2009). It has been hypothesized that shear rates within the mouth vary in response to the texture of a food product. (Janssen & others, 2007)) explain that there is an elongational component to flow in the mouth and that it may predominate over simple shear flow when breaking food structures to create a bolus. Furthermore, the same group observed that the addition of saliva to bolus formation increased the torque observed likely due to the breakdown of starch by salivary α-amylase and mechanical digestion.

1.3.10 Texture Profile Analysis

Texture profile analysis (TPA) is used to elicit information about textural characteristics of a food product upon application of a large-deformation force similar to oral processing (Szcześniak, 1962). Attributes such as chewiness, gumminess, hardness and cohesiveness are evaluated in relation to deformation under an applied force and measured objectively in terms of force applied, distance the force is applied through and time of deformation
(Burey & others, 2009). A sample of known dimensions is compressed uniaxially by a probe, released, and then compressed again to obtain a curve of compressive load versus time (Abd Karim, Norziah, & Seow, 2000) (Figure 3). Extensive work was conducted to establish definitions of textural attributes by Szczesniak (1962). Attributes related to semisolid dosage forms and therapeutic agent delivery during oral processing can be attained. Of note, hardness, defined as the maximum force applied in the first compression, can provide information regarding the force necessary to compress the dosage form between the tongue and palate or tongue and cheek. Cohesiveness is the strength of internal bonds and is a ratio of the maximum force or energy of the second compression to the maximum of the first compression. It will illustrate the behavior of the delivery system under deformation prior to rupturing. Gumminess is defined as the product of hardness and cohesive energy and is a measurement of the force required to completely disintegrate a food and subsequent bolus formation. The force required to completely remove a food product from oral surfaces is adhesiveness, given by the negative area under the curve after the first compression of the probe. The distance the food recovers after deformation is known as springiness and can be indicative of the durability of the confection within the oral cavity when deformed by the tongue or palate (Pons & Fiszman, 1996; Szczesniak, 1962; Abd Karim, Norziah, Seow, 2000; Sandhu & Singh, 2007).

Analysis of the impact of formulation changes or time-dependent changes on textural properties can also be realized with texture profile analysis. Siegwein, Vodovoz & Fisher
(2011) found that adding soy protein isolate to starch-based gummies, the hardness and cohesiveness of the confections decreased with increasing concentrations of protein. The study coincides with an investigation by Ribotta, Colombo, León & Añón (2007) in a similar study concluding that the addition of soy protein isolate will decrease the chewiness and gumminess of the gel.

![Figure 3. Typical curve from two-compression texture profile analysis and corresponding texture attributes (from Chen, 2009).](image)

1.3.11 Dissolution Testing with UV-Vis Spectroscopy

Dissolution testing is typically used in the Pharmaceutical industry to evaluate the release of a drug from a delivery form under standard conditions that mimic those found in the body. Performance of new therapeutic agents *in vivo* can be predicted first by dissolution analysis in terms of drug bioavailability (Azarmi, Roa & Löbenberg, 2007). The United
States Pharmacopoeia (USP) establishes standards for the dissolution apparatus and conditions used relative to the drug composition and target site of delivery. However, standards for new novel formulations may not exist. Oral dosage forms intended to deliver active ingredients to the oral cavity can be evaluated with dissolution testing. A dissolution apparatus for semisolid dosage forms for the oral cavity consists of a well, a chamber, and a water jacket to maintain body temperature. A drug release curve is generated by sampling the dissolution medium at precise time points relative to the rate of the dosage dissolution rate (Anand, Yu, Conner & Davit, 2011). Hughes (2008) designed a dissolution test to evaluate drug delivery to buccal tissue that utilized small volume, continuous flow-through filtration cells that mimicked the conditions within the oral cavity. Furthermore, dissolution testing of dosage forms for the oral cavity can be achieved in various media, specifically phosphate buffer, synthetic saliva formulations, or saliva from human or animal sources.

To quantitatively determine the performance of the drug delivery system by the pattern of drug release, chromatographic or spectroscopic methods are employed. It is common with plant polyphenols to use a liquid chromatography (LC) separation technique paired with a detector. Commonly used detectors include mass spectrometers, photodiode array detectors, and coulometric detectors, and sensitivity of these various detectors has been evaluated by Aaby, Ekeberg & Skrede (2007) and Määttä-Riihinen, Kamal-Eldin & Törrönen (2004). Plant polyphenols typically absorb in UV or visible light regions, and as a result can be characterized based on peak absorption at specific wavelengths (known
as $\lambda_{\text{max}}$ by photometric detectors. Of note, flavanols (catechin) absorb at 280 nm, hydroxycinnamic acid derivatives absorb between 300 -320 nm, ellagitannins at 350-360 nm and anthocyanins absorb between 500 – 520 nm, (Aaby, Ekeberg & Skrede, 2007; Mullen, Yokota, Lean & Crozier, 2003).

Use of the Folin-Ciocalteau colorimetry has been identified as a method for total phenolics determination in wines, tea, and fruits and vegetables (Waterhouse, 2002). The Folin-Ciocalteau reagent is a phosphomolybdate and phosphotungstate mixture that is oxidized readily in analysis solution. Phenolic compounds reduce the reagent by likely forming complexes with the molybdate species and produce a blue color. The ability of phenolics to inhibit oxidation allows for a measurement of total phenolics using spectroscopy to measure the saturation of blue color with a $\lambda_{\text{max}}$ of 765 nm (Waterhouse, 2002), assuming that a higher absorbance value indicates less oxidation of the reagent. A calibration curve using gallic acid is typically created for determination of phenolic amounts in the sample.

1.4 Strawberries

1.4.1 Production and Consumption

Strawberries are the edible fruits of the flowering plant of the family *Rosaceae* genus *Fragaria* (Bertelsen, Harwood, Hoff, Lee, Perez, Pollack, Somwaru, & Zepp, 1994). Although commonly referred to as “berries,” strawberries are actually the a part of the mature fruit known as the receptacle and the seed-like parts of the fruit are known as
achenes (Bertelsen & others, 1994). Strawberries are enjoyed by 94% of American households and are available year-round (California Strawberry Commission, 2009). The United States is the world’s largest strawberry producer and consumer with more than eight pounds of fresh and frozen strawberries available per capita (USDA Economic Research Service, 2011). The commercially-grown cultivars within the United States are from the hybrid *Fragaria x ananassa* (Darrow, 1966). In Ohio over 950 acres of land was farmed for strawberry production (Economic Research Service, 2009). It was reported that the value of strawberry production in Ohio was between 3.1 to 5.2 million dollars (NSF Center for Integrated Pest Management, 1999). Strawberry cultivars grown commercially within the United States are selected for berry size, high yield, stability during shipping and shelf life (Anonymous, 2006). Consumption of fresh strawberries is most common, with sales comprising 70% of total strawberry products. Frozen, pureed, and juiced preparations make up the remaining 30% of sales (Bertelsen, 1995). Many of the processed strawberry products are used in products such as jams, jellies, syrups, beverages, ice creams, yogurts, bakery products, and confectionery products (Anonymous, 2006).

1.4.2  Components and Stability

One hundred grams of whole strawberries contains approximately 32 kilocalories and is a good source of vitamin C (59 mg), fiber (2.0 g), potassium (153 mg), vitamin A (12 IU), folate (24 μg), contains low sugar (4.8 g), and contains carotenoids (β-carotene, lutein), phytosterols, and polyphenolics (anthocyanins, flavonols, hydroxycinnamic acid derivatives, and ellagitannins) (USDA National Nutrient Database for Standard
Reference; Aaby, Ekeberg & Skrede, 2007; Manach, Williamson, Morand, Scalbert & Remesy, 2005). The non-nutritive polyphenolic compounds found in strawberries (Figure 4) are of interest due to their health-promoting benefits and natural colorant properties, and as a result a minimal loss of these compounds during processing is desirable. The approximate content of strawberry phenolics is shown in Figure 4. Aaby, Skrede & Wrolstad (2005b) determined that strawberry achenes contributed 11% of total phenolics and 14% of the antioxidant activity. The total phenolics in strawberries at different maturation stages were studied by Wang & Lin (2000), and were found to be highest in small, green strawberries. Wang & Lin (2000) also showed that anthocyanin content, total phenolics, and antioxidant capacity varied significantly in different strawberry cultivars. Processing strawberries can lead to significant reduction in nutrient content and polyphenol degradation. Studies by Hartmann, Patz, Andlauer, Dietrich & Ludwig (2008a) showed that ascorbic acid content continuously decreased during processing and storage of strawberry juice and the highest loss occurred upon thawing. Polyphenols were found to be better retained in strawberry purees than juices. Polyphenolic compounds are subject to degradation by light, oxygen, heat, and enzymes. Drying methods such as lyophilization are used to maintain the polyphenolic content of berries as well as concentrate the health-promoting compounds (Michalczyk, Macura & Matuszuk, 2009; Stoner & others, 2010).

Flavonoids

Flavonoids present in strawberries include anthocyanins, quercetin, kaempferol, and catechins (Aaby, Skrede & Wrolstad, 2005a; Määttä-Riihinen, Kamal-Eldin & Törrönen
Flavonoids are monomeric constituents of condensed tannins (Bravo, 1998) typically present in a glycosylated form (Seeram, 2007). Anthocyanins are glycosylated hydroxyl or methoxyl derivatives of 2-phenylbenzopyrylium (anthocyanidin) or flavlyium ions (Mazza, Cacace & Kay, 2004) and differ in the number and position of methoxyl and hydroxyl groups on the anthocyanidin backbone, the position, identity, and type of glycoside moiety, and acylation of the glycoside (Wu & Prior, 2005). Strawberry anthocyanins mostly include Pelargonidin 3-glucoside (83%), Pelargonidin 3-rutinoside (8%), Cyanidin 3-glucoside (7%) (Wu & Prior, 2005). Flavonols quercetin and kaempferol are typically found in strawberries as quercetin-rutinoside, quercetin-glucoside, quercetin-glucuronide, and kaempferol-glucuronide (Seeram, 2006). Anthocyanin content was found to significantly increase as strawberries ripened (Wang & Lin, 2000). Anthocyanin stability is dependent upon the structure and the food matrix and composition in which they are found. Degradation occurs in the presence of heat, pH, light, oxygen, ascorbic acid, and oxidative enzymes (Hartmann, Patz, Andlauer, Dietrich & Ludwig, 2008a). Degradation pathways of strawberry anthocyanins differed at pH 1 and pH 3.5 upon heat treatment (Sadilova, Carle, Stintzing 2007). Polymerization of anthocyanins can occur with other phenolic compounds. The color of anthocyanins is also dependent upon pH. A red-colored flavylium cation exists in acidic aqueous environments (pH < 2), while a colorless hemiacetal predominates in a pH range of 2 – 4. A blue or purple-colored quinoidal base can be observed at a pH of 6 and increasing basic conditions yields an anionic structure (Giusti, Rodriguez-Saona & Wrolstad, 1999). Anthocyanins were significantly reduced upon juice extraction and pasteurization, but
every processing step in juice production was found to decrease the amount of anthocyanins (Hartmann & others 2008b). Similarly, processed foods containing strawberries in raw materials, such as jams and jellies, had undetectable levels of anthocyanins (Wu, Beecher, Holden, Haytowitz, Gebhardt & Prior, 2006).

Catechins
Catechin, a type of flavanol, was found in strawberries as isomers p-coumaroyl-glucoside and p-coumaroyl ester (Seeram, Adams Zhang, Lee, Sand, Schueller, & Heber, 2006; Määttä-Riihinen, Kamal-Eldin & Törrönen, 2004). Catechins are typically found in free forms rather than glycosylated forms (Törrönen & Määttä, 2002). Dimers, oligomers, and polymers of catechin molecules are known as proanthocyanidins, and upon application of heat and acid are hydrolyzed to anthocyanidins (Törrönen & Määttä, 2002).

Hydrolyzable Tannins
Hydrolyzable tannins are found as either ellagitannins or gallotannins, but the latter is not found in strawberries (Seeram & others, 2006). The structure consists of a polyol core (glucose or quinic acid) esterified with either gallic acids and/or hexahydroxydiphenic acids (HHDP) and is soluble in water (Seeram, 2006). Upon acid hydrolysis of ellagitannins, HHPD is released and ellagic acid is formed by joining two gallic acid molecules (Määttä-Riihinen, Kamal-Eldin & Törrönen 2004). The main sources of ellagic acid in the American diet are strawberries, raspberries, and blackberries (Wu & others 2006). Ellagitannins and ellagic acid were found to be the main contributors of antioxidant activity within strawberry achenes (Aaby, Skrede & Wrolstad, 2005a).

Hydroxycinnamic Acid Derivatives
Glucose and quinic acid esters of hydroxycinnamic acid have been identified in strawberries (Schuster & Hermann, 1985). The hydroxycinnamic acid derivative found in strawberries is $p$-coumaric acid (Määttä-Riihinen, Kamal-Eldin & Törrönen 2004).
Figure 4. Some polyphenolic compounds found in strawberries (from Seeram, 2007)
1.4.3 Strawberries and Health

Extensive work has been conducted to evaluate the role of fruits and vegetables in reducing the risk of chronic disease. Of particular interest to health are naturally-occurring non-nutrient phytochemicals in strawberries that exert metabolic effects that can promote health and decrease the risk of disease (Hannum, 2004; Stoner & others, 2007a). Epidemiologic, cohort, and case-control studies have assessed the correlations between fruit intake and the risk of cardiovascular disease (Andriambeloson, Magnier, Haan-Archipoff, Lobstein, Anton, Beretz, Stoclet & Andriantsitohaina, 1998; Dauchet, Amouyel, Hercberg & Dallongeville, 2006), stroke (He, Nowson, & MacGregor, 2006), cancer (Riboli & Norat, 2003; Pavia, Pileggi, Nobile & and Angelillo, 2006; Gonzalez, 2006), and neurodegenerative diseases (Galli, Shukitt-Hale, Youdim & Joseph, 2002), and have found it is significantly lower in populations consuming higher levels of

Figure 5. Approximate phenolic content of fresh strawberries (Määttä and Törrönen, 2000)
flavonoids (Ross & Kasum, 2002). There is extensive work showing the ability of polyphenolic phytochemicals in particular to protect the body against free-radical damage, aberrant cell proliferation and differentiation, and modulate enzymatic activity. As such, strawberries have great potential as chemopreventative agents in that their naturally-occurring phytochemical compounds have health-promoting properties that can prevent disease progression, malignancy, and recurrence (Seeram, 2008; Mallery, Zwick, Pei, Tong, Larsen, Shumway, Lu, Fields, Mumper & Stoner, 2008; Stoner & others, 2007b).

Antioxidant Activity

Antioxidant activity of strawberry polyphenols can act in concert with the body’s naturally occurring protection against damaging peroxyl, alkyl, superoxide, and peroxynitrite radicals (Kresty, Frankel, Hammond, Baird, Mele, Stoner & Fromkes, 2006; Basu, Wilkinson, Penugonda, Simmons, Betts & Lyons, 2009; Wang & Jiao, 2000). Polyphenolic compounds can readily donate a hydrogen atom from an aromatic hydroxyl group to a free radical (Ross & Kasum, 2002). Reactive oxygen species formed during aerobic metabolism can damage DNA, proteins, and lipids, and the accumulation of these unrepaired macromolecules can lead to the development of cancer, atherosclerosis, diabetes, and chronic inflammation (Ross & Kasum, 2002). It was found that strawberry extract increased the activity of antioxidant and oxidative stress repair enzymes (Zhang, Seeram, Lee, Feng & Heber, 2008). Strawberry flavonoids are also able to chelate metal ions and therefore prevent generation of reactive oxygen species. Flavonoids have been shown to inhibit lipid peroxidation within the phospholipid bilayer by localization within
the polar and nonpolar phases (Movileanu, Neagoe & Flonta, 2000). Quercetin has been
shown to increase glutathione S-transferase activity, an enzyme responsible for protecting
cells against oxidative stress (van Zanden, Ben Hamman, van Iersel, Boeren, Cnubben,
Lo Bello, Vervoort, van Bladeren & Rietjens 2003).

Antiproliferative Activity

Antiproliferative and anticancer activity of strawberry polyphenols has been
demonstrated by Seeram and others (2006). The use of freeze-dried strawberries as a
chemopreventative agent was found to inhibit tumor multiplicity and incidence in rat
esophageal cancer in 5% and 10% concentrations in chow (Stoner, Wang, Seguin, Rocha,
Stoner, Chiu & Kinghorn, 2010). Wang & Stoner (2008) demonstrated the
antiproliferative activity of strawberry extracts on human lung epithelial cancer cell lines.
Strawberry extract was found to decrease the activity of transcription factors involved in
tumor promotion mediators, specifically activator protein-1 (AP-1) and nuclear factor-κB
(NF-κB), in TPA- or UVB-induced tumor promotion as well as inhibited MAPK
hamster embryo cells was inhibited by freeze-dried strawberry extracts, especially in the
early cancer ‘initiation’ stage (Xue, Aziz, Sun, Cassady, Kamendulis, Xu, Stoner &
Klaunig, 2000). Xue and others (2000) hypothesized that the strawberry extract may
inhibit activation, DNA binding and repair, and/or detoxification in cells treated with
B[a]P. Seeram and others (Seeram & others, 2006) reported a dose-dependent anti-
proliferative effect in oral cell lines with a phenolic-enriched strawberry extract.
Inhibition of proliferation of humor oral tumor cell lines with both crude extracts and
isolated compounds from strawberries was reported by Zhang and others (2008). Lastly, it was found that strawberry extract was able to stimulate apoptosis in human HT-29 colon cancer cell lines (Seeram & others, 2006) and inhibit angiogenesis in a berry extract blend (Bagchi, Sen, Bagchi & Atalay, 2004).

Enzyme Modulation for Disease Prevention

Modulation of enzyme activity by strawberry extracts was shown by Seeram, Momin, Nair & Bourquin (2001). The extract inhibited cyclooxygenase enzymes COX-1 and COX-2 activity. Reddy, Alexander-Lindo & Nair (2005) showed cyanidin-3-glucoside extract inhibited COX-1 and COX-2, as well. Cyclooxygenase enzymes are responsible for the conversion of arachidonic acid to prostaglandins involved in inflammation, which is relevant to chronic disease etiology. Phase-II detoxification enzyme CYP1A1 was inhibited by strawberry extract (Kansanen, Mykkanen & Torronen, 1996). Ellagic acid was shown to increase glutathione-S-transferase, a detoxifying enzyme, in the liver (Das, Bickers & Mukhtar, 1985).

1.5 Oral Disease

1.5.1 Biology of Oral Disease

Oral diseases such as dental caries, periodontal disease, tooth loss, oral mucosal lesions, and oropharyngeal cancers, are the most common illnesses within the United States today (Wu, Beecher, Holden, Haytowitz, Gebhardt & Prior, 2006; Centers for Disease Control and Prevention, 2009). Recent research has linked oral disease with chronic diseases such as cardiovascular disease, kidney disease, and diabetes (Slade, Beck, Offenbacher, Heiss,
& Pankow, 2000); Kshirsagar, Moss, Elter, Beck, Offenbacher & Falk, 2005; Marigo, Cerreto, Giuliani, Somma, Lajolo & Cordaro, 2011). Oral maladies and noncommunicable chronic disease share common risk factors that may be interrelated, including diet, tobacco use, alcohol use, and socioeconomic status. Overall well-being and quality of life may be compromised due to difficulty chewing, eating, speaking, and subsequent loss of self-esteem (Hollister & Weintraub, 1993). Many oral diseases are easily preventable with simple daily dental hygiene regimens, routine dental exams and modifiable behaviors. For example, tobacco use is a known risk factor for oral cancer, oral mucosal lesions, and periodontal disease (Winn, 2001). However, the existence of health disparities leads to inadequate dental care and inequalities in clinical outcomes (Centers for Disease Control and Prevention, 2011). As a result, the need for a clinically effective oral disease prevention and treatment methods persists.

The etiology of dental caries and periodontal diseases is directly associated with the accumulation of bacterial plaque on teeth, and untreated tooth decay can result in pain, dysfunction, and tooth loss (Centers for Disease Control and Prevention, 2011). In comparison to global incidence, the United States has a moderate level of dental caries amongst 35-44 year olds (Petersen, Bourgeois, Ogawa, Estupinan-Day & Ndiaye, 2005) and 96% of adults between 50-64 year olds have reported having dental caries (Centers for Disease Control and Prevention, 2011). Periodontal disease affects between 4-12% of American adults and is the result of inflammation (Centers for Disease Control and Prevention, 2011). Oral mucositis is an inflammatory condition resulting mainly from
cancer treatment therapies (Sankar, Hearnden, Hull, Juras, Greenberg, Kerr, Lockhart, Patton, Porter & Thornhill, 2011). According to the National Cancer Institute, more than 36,500 men and 11,000 women will be diagnosed with oral cancer in the United States and 7,880 will die from the disease in 2010 (Howlader, Noone, Krapcho, Neyman, Aminou, Waldron, Altekruse, Kosary, Ruhl, Tatalovich, Cho, Mariotto, Eisner, Lewis, Chen, Feuer, Cronin, Edwards (eds). Squamous cell carcinomas account for nearly 90% of oral cancers (Sankar & others, 2011). It has been established that tobacco use accounts for nearly 90% of oral cancers, and when combined with alcohol use the relative risk of developing oral cancer increases by 30 (Petersen & others, 2004). Additional risk factors for oral cancer include gastroesophageal reflux disease, poor nutrition, human papilloma virus, Epstein-Barr virus, betel seed chewing, immunodeficiency, certain workplace exposures in metalworking, refining, and textiles, and certain genetic predispositions (Sturgis, Wei & Spitz, 2004; Centers for Disease Control and Prevention, 2009). The protective effects of fruit and non-starchy vegetables has been identified as conferring probably decrease in relative risk (World Cancer Research Fund / American Institute for Cancer Research). The five-year survival rate for early-stage disease is approximately 80% but drops significantly to 9% in late-stage disease prognosis (Centers for Disease Control and Prevention, 2009). Salivary hypofunction and xerostomia result in reduced salivary fluid volume and/or a change in whole saliva composition yielding persistent dry mouth (Sankar & others, 2011).

1.5.2 Topical Agents for Treatment and Prevention
The prevention and treatment of oral disease is relative to the type of oral malady. Superficial diseases such as caries and plaque require only topical agents, yet are still limited by the dynamic environment of the oral cavity. Palliative therapy to augment salivary hypofunction and xerostomia rely upon the use of gustatory agents, saliva substitutes, and lubricants to aid in saliva stimulation (Sankar & others, 2011). Rinses, gels, and troches have been identified as potential treatment agents. Diseases of the epithelium such as oral dysplasia and mucositis require therapeutic agents to penetrate the cell membrane and be retained by the epithelial tissue (Squier & Kremer, 2001). Barrier function in diseased individuals is reduced and therefore facilitates easier uptake, yet can also readily lose therapeutic agents. For example, Sankar and others (2011) explain that non-lesional areas surrounding leukoplakia showed significantly higher permeability than normal oral mucosa. Oral lesions and pre-cancers require agents that target the aberrant biological pathways that lead to dysplasia and eventually cancerous tumors. Topical treatments such as mucoadhesive discs and gels and oral rinses have been employed as drug delivery agents (Sankar & others, 2011). Traditionally, oral squamous cell carcinomas are treated with tumor excision, radiotherapy, or chemotherapy or a combination thereof (Sankar & others, 2011). Chemotherapeutic agents and radiation therapy yield ulcerated epithelium that is limited in its proliferative ability and can subsequently increase the ability of a therapeutic agent to remain in contact with the diseased tissue (Squier & Kremer, 2001). Therapeutically effective treatment of oral dysplasia likely requires prolonged contact between the therapeutic agent and the
diseased cells. Prevention of pre-cancer and cancer may even begin long before dysplastic cells arise.

1.5.3 Semisolid Dosage Forms

The uptake and bioavailability of strawberry bioactives is dependent upon the molecular structure of the compound and the food matrix composition in which it is ingested. Delivery of bioactives requires the food matrix to provide patients with sustained, targeted release of biologically-active compounds. It is therefore critical to identify the target tissue and the feasibility of the dosage form to deliver a therapeutically effective dose. The release of therapeutic agents from gel systems and the clinical efficacy of treatment depend inherently on the drug release and the rheological, textural, and clinical properties of the formulation (Bruschi, Jones, Panzeri, Gremião, de Freitas & Lara, 2007). Topical applications must exhibit good retention at the site of application, must have low hardness or require low shear, and must have an easy application (Jones, Lawlor & Woolfson, 2004). It is typically understood that aqueous-based formulations such as mouthwashes, aerosols, and suspensions, have poor retention within the oral cavity (Jones, Woolfson & Brown 1997). A mouthwash containing a cyclooxygenase inhibitor to prevent oral cancer in patients with leukoplakia was evaluated in a clinical study, and while effective in benchtop analyses, did not show a significant tissue penetration when used in vivo (Mulshine, Atkinson, Greer, Papadimitrakopoulou, Van Waes, Rudy, Martin, Steinberg, Liewehr, Avis, Linnoila, Hewitt, Lippman, Frye & Cavanaugh, 2004). Additionally, non-aqueous formulations, such as gums, patches, and
ointments, are readily retained within the oral cavity yet do not offer a desirable mouthfeel or ease of use (Jones, Woolfson & Brown, 1997).

Semisolid dosage forms do, however, confer a more desirable mouthfeel and coat the mouth to more readily increase the retention time within the oral cavity (Salamat-Miller, Chittchang & Johnston, 2005). Gel formulations have been proposed as a means of improving compliance of dysphagic and geriatric patients who have difficulties handling or taking oral dosage forms (Miyazaki, Takahashi, Itoh, Ishitani, Dairaku, Togashi, Mikami & Attwood, 2009). The use of a topically-applied gel containing 5% and 10% freeze-dried black raspberries has been evaluated in vivo for chemopreventive activity against oral epithelial dysplasia by Mallery and others (2008). The study concluded that the berry gel was non-toxic and delivered clinically-therapeutic doses of black raspberry bioactives after 6 weeks of treatment.

1.6 Oral Drug Delivery

1.6.1 Oral Cavity

The action of a drug delivery system is defined by the administration, release, and delivery of a therapeutic substance by a specific formulation that modulates the rate, time, and site of action (Jain, 2008). Pharmacologic effects in oral tissue by local drug delivery systems are partly determined by the anatomy and physiology of the mouth. The surface area of the moist tissue lining the oral cavity, or oral mucosa, is approximately 100 cm² (Sandri, Bonferoni, Ferrari, Rossi & Caramella, 2006) and acts as a protective
barrier to xenobiotics, reduces shearing forces during mastication, and provides sensations during speech and eating (Squier & Kremer, 2001). The oral cavity is comprised of three types of mucosa: masticatory, lining, and specialized (Squier & Kremer, 2001). The non-keratinized stratified squamous epithelium of the lining mucosa accounts for 60% of the total surface area and is more permeable than the keratinized epithelium; therefore, it provides an ideal route of local drug delivery (Squier & Kremer, 2001; Hao & Heng, 2003). Specifically, the lining mucosa covers tissue on lips, cheeks, soft palate, the floor of the mouth, the ventral surface of the tongue and vestibule, as depicted in Figure 6 (Sankar & others, 2011). Among the various types of lining mucosa, buccal cells the most common site for local treatments.

The dynamic environment of the oral cavity presents limitations to local drug delivery and must be considered during development of a potential therapeutic agent. A mucus layer with a thickness ranging from 1 to 400 µm coats the buccal mucosa and acts as a protective barrier, thus limiting drug permeation (Hao & Heng, 2003). Permeability also changes as buccal mucosal cells turnover. Studies by Thompson, Potten, and Appleton (2001) revealed cell turnover after approximately fourteen days, but has also found to be as few as every three to eight days (Hao & Heng, 2003). Dosage forms for oral delivery are also limited by the taste, dose, and mechanical stress imposed upon the patient (Sankar & others 2011). Additionally, person to person variation in the oral cavity environment can alter the delivery and uptake of a therapeutic agent. Variations in the surface area, enzymatic degradation, oral microbiota, saliva production, oral disease, and
compliance contribute to the obstacles of oral drug delivery (Hao and Heng, 2003; Sankar & others, 2011).

Figure 6 Mucosa of the oral cavity (Spierer & Kremer, 2001)

1.6.2 Saliva

Saliva plays a key role in drug release and delivery within the oral cavity, especially when using food-based treatments. Saliva is a heterogeneous aqueous liquid that provides lubrication and solubilization to facilitate bolus formation during eating, initiates digestion of carbohydrates and fats, and confers protective effects through antibacterial activity and buffering action ((Mandel, 1974; Chen, 2009). Approximately 98% of whole saliva is water, with the remaining 2% is comprised of electrolytes (Na\(^+\), Cl\(^-\), HCO\(_3\)\(^-\), and K\(^+\)), mucus, glycoproteins, enzymes, and antibacterial compounds (Chen, 2009). The pH of saliva ranges between 5.6 – 7.6 in healthy individuals (Chen, 2009). Saliva exhibits shear thinning behavior likely due to the presence of mucin glycoproteins (Schipper,
The glands responsible for secreting saliva are the parotid gland, sublingual gland, and submandibular glands, and each gland secretes a different component of whole saliva (Speier and Kremer, 2001). Parasympathetic saliva secretion can occur without a stimulus, yielding watery, copious saliva. Sympathetic saliva secretion results upon response to many different stimuli and yields more viscous, protein-rich saliva (Speier and Kremer, 2001).

The viscosity of parotid saliva upon stimulation by food was found by Prinz, Wijk, and Hunterin (2007) to be 1.0 mPa*s, and that of sublingual and submandibular saliva was found to be 2.1 mPa*s. Less viscous, mucin-rich saliva from the sublingual and submandibular glands was the main component of whole saliva in the absence of food (Prinz, Wijk, Hunterin, 2007). Additionally, the flow rate of saliva is dependent upon the type of nervous system regulation and stimulus as well the individual. Unstimulated saliva flow in healthy adults was approximately 0.1-0.2 mL/min whereas stimulated saliva flows at a rate of up to 7 mL/min (Donovan, 2005). Chen (2009) found stimulated saliva flow to be at the highest rate in the early morning and around noon, but unstimulated saliva was not shown to change throughout the course of the day.

1.6.3 Saliva Functionality and Flow

The ability of saliva to aid in food digestion and bolus formation upon eating is partly due to the proteins within whole saliva that initiate carbohydrate hydrolysis and reduce stress during oral processing. Mucins, large glycoproteins, facilitate oral processing by creating a viscoelastic mixture that requires less stress to deform (Schipper & others, 2008).
Salivary α-amylase hydrolyzes the 1-4 glucosidic bonds between glucose moieties in amylose fractions of starch to the simple sugars maltose, maltriose, and α-limit dextrins (Schipper, Silletti & Vingerhoeds, 2007). The optimum pH of salivary α-amylase is 6.9, and the rate of hydrolysis is dependent upon the oral cavity environment and the food matrix (Chen, 2009). The activity of α-amylase shows a diurnal pattern, with a steady increase in activity throughout the course of the day beginning about sixty minutes after waking (Nater, Rohleder, Schlotz, Ehlert & Kirschbaum, 2007). Gender, body mass, physical activity, cigarette smoking, and food and drink consumption prior to amylase measurement did not show a marked change in activity throughout the day, yet increased age showed an attenuated diurnal pattern in saliva secretion (Nater, Rohleder, Schlotz, Ehlert & Kirschbaum, 2007). However, since salivary α-amylase secretion is the result of sympathetic nervous system signaling via B-adrenergic receptors, levels of the enzyme may be indicative of stress levels. Nater & others (2007) found that long-term stress had a more pronounced increase in daily salivary amylase levels than temporal or perceived momentary stress. Furthermore, Klein, Bennett, Whetzel, Granger & Ritter (2010) found that acute stress paired with caffeine consumption was responsible for increased salivary α-amylase levels when compared to placebo. Chronic inflammation can also alter saliva flow and α-amylase levels. Saliva constituents and whole saliva flow rates are altered in periodontitis patients (Sanchez, Miozza, Delgado, Busch, 2011). Additionally, differing reports on the impact of cigarette smoking have shown that in some instances smoking will decrease the activity of α-amylase yet others have shown that cigarettes do not alter activity (Nater & others, 2007). Lastly, racial differences due to polymorphisms from
salivary amylase alleles have been shown to alter amylase functionality and are reviewed by Azen (1993).
Chapter 2: Characterization of Physical Properties and Effects of Time-dependent Changes and \textit{in vitro} Dissolution Kinetics of a Novel Strawberry Confectionery

2.1 Introduction

Oral health has a significant effect on overall health and quality of life (Gift & Atchison, 1995). Oral diseases are a common health concern in the United States, and studies have revealed that poor oral health may lead to increased risk of certain chronic diseases (Wu, 2009). Conditions such as periodonitis, xerostomia, mucositis, and tooth decay are associated with co-morbidities such as decreased saliva production, difficulty chewing and swallowing, and loss of taste (Gift & Atchison, 1995). In addition, new diagnoses of oral cancer are estimated to be in excess of 35,000 in 2010 (Centers for Disease Control and Prevention, 2009). The association of chronic disease and oral health is possibly due to infection, chronic inflammation, genetic predisposition, and potentially nutrition (Ritchie, Joshipura, Hung & Douglass, 2002; Ritchie, Smith, Summersgill, Hoffman, Wang, Klusmann, Turek & Haugen, 2003).

Fruit phenolics have been shown to elicit significant protective effects on oral mucosa when evaluated in numerous pre-clinical animal models (Seeram, 2008, Stoner, Wang, Zikri, Chen, Hecht, Huang, Sardo & Lechner, 2007a; Stoner, 2009) and may be a novel
prevention tool instead of costly pharmaceutical agents that may have undesirable side effects. Strawberry (Fragaria x ananassa) phytochemicals, such as vitamin A, vitamin E, ascorbic acid, ellagitannins, anthocyanins, flanorols, and catechins, confer known anti-inflammatory, anti-oxidant, and anticarcinogenic properties that could improve associated oral maladies (Zafra-Stone, Yasmin, Bagchi, Chatterjee, Vinson & Bagchi, 2007; Hamalainen, Nieminen, Vuorela, Heinonen & Moilanen, 2007; Palacios, Joshipura & Willett, 2009; Seeram, 2006; Wang, Feng, Lu, Bowman & Ding, 2005).

Although gels, gums, and rinses can be effective vehicles of therapeutic agent delivery, the limitations in taste, dose, and mechanical stress imposed by these delivery models pose a challenge in individuals with loss of teeth, oral sores, and other oral maladies (Madhav, Shakya, Shakya & Singh, 2009, Zhang, Zhang & Streisand, 2002). Therefore, a soft confection consisting of fruit phytonutrients from which bioactive compounds can be easily liberated from its starch matrix without mastication may be desirable in delivering bioactive ingredients to the oral mucosa. A unique starch-based confection containing freeze-dried strawberries was developed to provide sustained, targeted release of phytochemicals to the oral mucosa to promote oral health in a phase I/II clinical trial.

The biological efficacy of a semisolid dosage form in part relies upon the physicochemical properties of the food matrix (Hao & Heng, 2003). It is imperative that the food matrix maintains an environment suitable for the phytochemicals of interest. That is, many fruit bioactives are readily degraded and therefore the proper pH, moisture
content, and solute concentration must inhibit or slow degradation of the target compound (Klopotek, Otto & Bohm, 2005, Sadilova, Carle & Stintzing, 2007). In order to prolong contact between the fruit phytochemicals and the oral mucosa, the semisolid dosage form must be easily administered, not rapidly disintegrate, and impart pleasant taste and texture. Manipulation of specific physical and chemical properties of a food system for targeted bioactive delivery can be achieved, and requires instrumental characterization of such properties. Physicochemical analysis of the confection was conducted to determine textural properties, such as cohesiveness, adhesiveness, and hardness, as well as to characterize the rheological behavior of the gel microstructure. *In vitro* dissolution kinetics in artificial saliva was elucidated to provide a profile of bioactive release from the confectionery delivery form. Time-dependent changes such as starch retrogradation and the effects on physical properties in the food microstructure were evaluated. The objective of this study was to compare physicochemical properties of the confection with *in vitro* dissolution studies to determine the facility of the confection to sustain phytochemical release in the oral cavity and to quantify time-dependent changes in the confection upon storage.

2.2 Materials and Methods

2.2.1 Confection Formulation

Optimized starch-based confections were made by mixing modified corn starch (Confectioners G, Tate and Lyle, USA), corn syrup (Gordon Food Service, USA), and distilled water over heat in amounts shown in Table 1. The mixture was stirred and
heated on a hot plate until a gel was formed as indicated by °Brix between 65 to 68 and a final temperature of 90 °C, typically achieved between 10-14 minutes. Upon cooling to 60 °C at ambient room temperature, freeze-dried strawberry powder (FDS) (*Fragaria x ananassa*, California Strawberry Commission, Watsonville, CA) was mixed into the gel. The mixture was deposited at 40 °C with a pastry bag onto a silicon mat. Control confections (no strawberry powder) were made by the same process with a formulation containing sugar-free strawberry drink powder (Kool-Aid, Kraft Foods, Inc., US) (Table 1). Confections were cut into six gram pieces and were stored in plastic two ounce cups with lids and kept at room temperature out of light for at least twenty four hours prior to packaging. Measurement of pH was obtained for confections upon packaging with a pH meter (Mettler Toledo, US). The pH of the FDS confections was measured to be between 3.1-3.4. The caloric content of a six gram confection was determined to be 15 kcal/gram by calorimetric analysis (Bomb Calorimeter, Parr Instruments, Moline, IL). A confection sample between 0.6-0.8 grams removed centrally from a six gram candy was used for analysis. 2000 mL distilled water was used in the adiabatic water bath chamber (Parr Instruments, Moline, IL). Oxygen (30 atm) was put into the bomb chamber (Parr Instruments, Moline, IL). The bomb was equilibrated to 24 °C and upon ignition, the temperature was monitored with a glass thermometer. A benzoic acid standard was used for calculations. Samples were analyzed 24-hours after curing. Time-dependent changes were determined from samples after an additional seven and fourteen days of storage.
Table 1. Confectionery formulations for study agents in clinical trial

<table>
<thead>
<tr>
<th>FDS Confections</th>
<th>% Wet Basis</th>
<th>Control Confections</th>
<th>% Wet Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeze-dried fruit powder</td>
<td>48.5</td>
<td>Corn Syrup</td>
<td>49.2</td>
</tr>
<tr>
<td>Corn Syrup</td>
<td>24.3</td>
<td>Modified Corn Starch</td>
<td>24.6</td>
</tr>
<tr>
<td>Modified corn starch</td>
<td>10.1</td>
<td>Water</td>
<td>25.2</td>
</tr>
<tr>
<td>Water</td>
<td>17.1</td>
<td>Flavor</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

2.2.2 Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was used to identify different water as a result of mass loss upon heating the confections. A TGA Q5000 (TA Instruments, New Castle, DE) was used to analyze 15-20 mg samples of confections at a heating rate of 10 °C/min from 20 – 200 °C with a Hi-Res function at 4 °C/min in regions of rapid mass loss. TA Universal Analysis software (TA Instruments, New Castle, DE) was used to integrate peaks of mass loss to determine moisture content. Three replicates of samples were analyzed.

2.2.3 Texture Profile Analysis

An Instron 5542 (Instron Corporation, Canton, MA) affixed with a 35 mm steel parallel plate compressed 6 gram cylindrical confection samples at a rate of 30 mm/sec to 30% deformation with two compressions. Attributes obtained were hardness (N), cohesion energy, gumminess (N), adhesiveness (J), chewiness (N), springiness (mm), and cohesion
force. At least eight replications were performed, as the large variation between samples can occur in texture analysis.

2.2.4 Rheological Analysis

Rheological testing of strawberry confections was conducted with an AR 2000ex Controlled Stress Rheometer (TA Instruments, New Castle, DE) affixed with a 10 mm steel parallel plate and a 1.0 mm gap. To elicit information regarding the viscoelastic behavior of the confections under applied stress, oscillatory frequency sweeps were used from 0.01 – 100 Hz, 25 and 37 °C at a stress within the linear viscoelastic region established from an oscillatory stress sweep (data not shown). The viscous modulus (G’), elastic modulus (G’’) and complex viscosity (ƞ*) were monitored throughout the sweep with Rheology Advantage Data Analysis v5.7.0 software from TA Instruments. Temperature-dependent changes in the response to deformation were assessed with a temperature ramp from 20 to 50 °C, 1 Hz and a stress from the linear viscoelastic region. Apparent viscosity (ƞ) at increasing shear rates was determined from flow analysis within a range of 0.01 – 1000 s⁻¹ and 1 Hz at 37 °C. Oscillatory analyses were performed in triplicate and flow analysis was performed in duplicate.

2.2.5 Dissolution Testing

Bioactive release from the starch-based confectionery matrix was evaluated with a VanKel VK 7010 dissolution testing station (VanKel Technology, Cary, NC, USA) with USP apparatus I in a synthetic saliva solution adapted from Tavss, Gaffar, and King (1984) with porcine pancreatic α-amylase and porcine gastric mucin (Sigma, USA). One
six-gram confection post-curing was placed in a rotating basket (100 RPM) and submersed in 250 mL of media at 37 °C. Sampling of 5 mL of the dissolution medium occurred at evenly spaced intervals and was replaced by medium upon sampling. Samples were acidified with formic acid (5% v/v) and frozen at -25 °C prior to analysis. A beaker containing 250 mL of acidified simulated saliva solution was kept at room temperature as a control to determine the percent release of fruit bioactives. Release of total phenolics was evaluated using microscale Folin-Ciocalteau colorimetry with gallic acid as the standard (Current Protocols in Food Analytical Chemistry 2002). Total monomeric anthocyanins released were evaluated by UV-Vis spectroscopy (UV-2401PC Spectrophotometer, Shimadzu, Japan) with the protocol developed by Rodriguez-Saona, Wrolstad, and Giusti (2002).

2.2.6 Storage Study

Confection forms for the clinical trial were batch-processed, which necessitates an investigation into the stability and functionality of confections upon storage prior to use. Starch-based food products are known to undergo physical changes upon storage, and therefore characterization of the physical properties was also conducted to evaluate time-dependent changes by aforementioned methods at day 1, day 7, and day 14 of storage (page 47-48). Thermogravimetric analysis was used to investigate changes in water mobility and moisture content in confections pre-cure, twenty-four hours post-cure, seven days and fourteen days of storage. Texture profile analysis was conducted to identify changes in organoleptic properties of the confections. Lastly, Changes in the confection
microstructure in response to deformation were evaluated with oscillatory frequency sweep and flow analysis.

2.2.7 Statistical Analysis

A Kruskal-Wallis one-way analysis of variance (ANOVA) was used to determine differences between median values on storage days per texture attribute (p<0.05). A post hoc analysis with Tukey’s HSD to identify which groups differed from one another when a significant difference was found. Analysis was accomplished with SigmaPlot v.11 (Systat Software, Inc., USA).

2.3 Results and Discussion

2.3.1 Thermogravimetric Analysis

To determine the moisture content and water behavior of the strawberry confections, thermogravimetric analysis was used. The percentage of mass lost up to 150 °C was monitored and assumed to be water (Figure 7A), indicating strawberry confections had 22.3% moisture. One defined water population was observed, as indicated by a region of accelerated mass loss (Figure 7B). Mass lost above 150 °C was assumed to be due to burning.
2.3.2 Texture Profile Analysis

Texture attributes were quantified from a TPA curve (Figure 8). The curve obtained by TPA showed peaks typical of maize starch gels. Median and mean values for day 1 analysis are presented in Table 2. Small peaks near the maximum compressive load are indicative of factorability and are typical of food gels (Karim & Bhat, 2008). Based on the springiness and hardness values, it is reasonable to conclude that the confections can maintain structural integrity within the oral cavity during oral processing, assuming the consumer is not chewing or biting the confection (per directions for participants in the clinical study). Oral processing of semisolid food systems was studied by Arai & Yamada (1993), and revealed that below a threshold of 0.08 kg (force) for agar gels and 0.03 kg for gelatin gels, oral processing changes from compression between the tongue and hard palate to dentition. Additionally, Hiemae & Palmer (1999) explained that the time of
bolus formation increases with increasing hardness. While TPA provides important information regarding the behavior of food products undergoing large deformation, additional changes occur with saliva and tongue manipulation that are not quantified by TPA.

![Graph showing compressive load over time](image)

**Figure 8.** Compressive load over time for determination of texture profile attributes. Graph shown is median of eight replicates

### 2.3.3 Rheological Analysis

Characterization of the confectionery microstructure response to deformation was performed with an oscillatory frequency sweep and flow testing. Increasing oscillatory frequency resulted in an increase in both the elastic modulus (G’) and the viscous modulus (G”) both at 25 and 37 °C as shown in Figure 9. The elastic modulus remained greater than the viscous modulus over the range of frequencies tested at both 25 and 37 °C, showing confections exhibited more solid-like properties in response to deformation and was not greatly influenced by room temperature or human body temperature. Weak gels are characterized by continuously higher G’ values, and therefore the confectionery
gel system can be described as a weak gel (Tabilo-Munizaga & Barbosa-Cánovas, 2004). The complex viscosity ($\eta^*$) decreased with increasing frequency (Figure 9). Correlation between texture perception and frequency were found to be greatest at values near 50 rad/s (Malone, Appelqvist, & Norton, 2003), but oral processing can involve frequencies between 0.1-100 Hz (Chen, 2009).

Further characterization of the gel structure up deformation was achieved by investigating the flow behavior of the confection with increasing shear. In non-Newtonian materials, the apparent viscosity is shear-dependent and decreases with increase shear in a thinning or pseudoplastic pattern (Nguyen, Jensen & Kristensen, 1998). It was found that within the range of shear evaluated, the confection exhibited both Newtonian-like flow and psuedoplastic flow typical of shear-thinning semi-solids from biopolymers (Figure 10) (Rao, 1998). Shear rates below 10 sec$^{-1}$ were associated with linear, Newtonian-like decrease in viscosity. Changes in molecular rearrangement at approximately 10 sec$^{-1}$ is indicative of a shift to pseudoplastic behavior as presented in Figure 10, and the reciprocal of the shear rate at which this change occurs is referred to as the time constant (Rao, 1998). Textural attributes were found to correlate well with shear rates between 5-100 s$^{-1}$ (Malone, Appelqvist, & Norton, 2003).

Foods are exposed to a change in temperature when ingested and as a result rheological behavior may change. Figure 11 presents the temperature-dependent changes in the confectionery. It was found that a decrease in elastic and viscous moduli occurred and...
was most pronounced in the range of 20 to 30 °C. The slight decrease in the moduli value likely will not impact oral processing or bioactive delivery.

Figure 9. Oscillatory frequency sweep investigating FDS confections under deformation at 37 °C (A) and 25 °C (B). Symbols represented elastic modulus (●), viscous modulus (○), and dynamic viscosity (x)
Figure 10. Strain-dependent response of apparent viscosity in FDS confection at 37 °C.

Figure 11. Temperature dependence of elastic (solid triangle) and viscous moduli (open triangle)

2.3.4 Dissolution Testing
Release of strawberry bioactives from the starch-based confectionery matrix was investigated by dissolution testing. Release of total phenolics was found to reach 86% after six hours (Figure 12). Total monomeric anthocyanin release achieved an 83% release after six hours (Figure 13). Slow release of bioactives could be explained by Vesterinen Myllärinen, Forssell, Söderling & Autio (2002), who showed a strong correlation between the storage modulus (G’) and the extent of maize starch hydrolysis by salivary α-amylase in vivo and in vitro. That is, more rigid starch gels exhibited a lesser percentage of reducing sugars, indicating that the salivary α-amylase was unable to react with the starch substrate due to decreased porosity of the more rigid starch network. Work conducted in our laboratory reflected the association between a larger elastic modulus and a prolonged dissolution time of confectionery products (Ahn-Jarvis, Schwartz, Weghorst & Vodovotz, unpublished). Starch-based grape juice confections with less solid-like behavior modulated by soy protein isolate addition were shown to have a more rapid dissolution as compared to more solid-like starch-only confections (Ahn-Jarvis, Schwartz, Weghorst & Vodovotz, unpublished). The slow release of the strawberry confections establishes the potential as a novel delivery system that can provide sustained delivery of bioactives to the oral mucosa without rapid disintegration or loss of product to swallowing.

Complete bioactive release and confection digestion may have been impeded by several factors. Starch-based low moisture foods swell when placed in aqueous solutions, including saliva (Smith, 1999), and swelling of the starch-based confection within the
submersion basket may have impeded complete dissolution and total release of the fruit bioactives. Additionally, other non-starch polysaccharides, proteins, and the strawberry achenes may have created a more digestion-resistant food matrix by forming complexes with phenolics inhibiting amylase-starch reactions.

Figure 12 Release of total phenolics in simulated saliva solution from strawberry confection
2.3.5 Storage Study

Confections analyzed pre-curing contained 24% moisture, day 1 had 22.3% moisture, day 7 had 19.3% moisture, and day 14 had 18.2% moisture (Figure 14A). Confections after storage showed one temperature region of accelerated mass loss between 90 – 120 °C, whereas confections immediately after manufacture showed multiple water populations, as indicated by several temperature ranges of accelerated mass loss (Figure 14B). It was observed that upon storage the rate of moisture loss shifted to higher temperature ranges as shown in Figure 14B. The shift in moisture loss at higher temperatures indicated the water remaining is more tightly bound (Tian, Li, Xu & Jin, 2011). Unbound water during starch retrogradation can be expected to be more readily lost as starch side chain interactions increase. Hydrophobic regions within the crystalline portions of the retrograded starch also decrease water-starch interactions over time (Tian, Li, Xu & Jin, 2011). As amylopectin side chains recrystallize, bound water increases but is not readily lost.

Large deformation testing of the confections revealed changes in textural properties upon storage. Specifically, confections stored for fourteen days were significantly harder, gummier, and chewier than confections at day 1 and showed a larger range of deviation from the mean value (Table 2). It was expected time-dependent changes would occur as the amylopectin side groups recrystallize and become more rigid, and therefore require more force to compress the confection. Furthermore, TGA revealed less moisture in the
food system at day fourteen, which may lead to more rapid starch re-ordering and a significant increase in hardness (Roos, 1995). Springiness did not show significant differences during storage between day 1 and 14, yet day 7 was significantly different from day 14, implicating an attribute of the food matrix that is not time-dependent. It is likely that air pockets formed within the confectionery matrix were maintained throughout storage and were responsible for the springiness values observed throughout the compression cycles. A rigid outer skin that appeared by day 14 may have contributed to the recovery after deformation, as well. Adhesiveness and cohesiveness values did not differ during storage, and are likely indicative of a skin or dry, hard exterior that is known to form on sugar confections upon loss of moisture (Ergun, Lietha & Hartel, 2010).

Amylopectin recrystallization during storage was apparent in the rheological analysis as the storage modulus increased over time, which indicated an increase in solid-like properties. Rheological trends in starch retrogradation reflect published data for corn starch gels by Yoshimura, Takaya & Nishinari (1998) that indicated more solid-like behavior and concomitant increase in moduli with an increase in frequency and shear. Similarly, Farhat, Blanshard & Mitchell (2000) used X-ray diffraction to demonstrate that an increase in solid-like components and hardness of starch gels during storage can be correlated to an increase in crystallinity, more specifically of amylopectin recrystallization. The onset of shear-thinning behavior in confections stored occurred at lower shear rates than day 1 confections.
Figure 14. Thermogravimetric analysis of moisture loss (A) and rate of moisture loss (B) in strawberry confections during storage. Colors denote confections pre-cure (black), day 1 (dark pink), day 7 (red), and day 14 (pink).
Table 2 Changes in texture profile of confectionery over storage time. Values shown are the median and mean ± standard deviation; n=8. Symbols indicate significant difference between storage days per attribute calculated from the median.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>Hardness (N)</td>
<td>5.53</td>
<td>5.89±2.32</td>
<td>7.88</td>
</tr>
<tr>
<td>Cohesion Energy</td>
<td>0.595</td>
<td>0.595±0.0304</td>
<td>0.556</td>
</tr>
<tr>
<td>Gumminess (N)</td>
<td>3.54</td>
<td>3.50±1.22</td>
<td>4.38</td>
</tr>
<tr>
<td>Adhesiveness (J)</td>
<td>7.50E-04</td>
<td>8.20E-04±4.3 E-04</td>
<td>9.30 E-04</td>
</tr>
<tr>
<td>Chewiness (N)</td>
<td>13.3</td>
<td>13.1±4.56</td>
<td>16.4</td>
</tr>
<tr>
<td>Springiness (mm)</td>
<td>2.39†‡</td>
<td>2.38±0.132</td>
<td>2.73†</td>
</tr>
<tr>
<td>Cohesion Force</td>
<td>0.820</td>
<td>0.850±0.0636</td>
<td>0.810</td>
</tr>
</tbody>
</table>
Figure 15 Large deformation testing reflects time-dependent changes in confectionery at day 1 (solid line), day 7 (dashed line), and day 14 (dotted line)
Figure 16 Frequency dependence of elastic and viscous moduli in strawberry confections at 37 °C during storage. Symbols denote day 1 (▲), day 7 (●), and day 14 (■) with filled symbols for G’ and unfilled symbols for G’’.
Figure 17 Decrease in complex viscosity of strawberry confections at 37 °C during storage. Symbols denote day 1 (▲), day 7 (●), and day 14 (■).
Figure 18 Decrease in viscosity of strawberry confections 37 °C with increasing shear rate. Symbols represent day 1 (▲), day 7 (●), and day 14 (■).

2.4 Conclusion

Characterization of a novel starch-based confectionery containing freeze-dried strawberry powder was achieved. Thermogravimetric Analysis revealed the moisture content of the confections decreased upon storage and water within the system remaining after fourteen day storage was more tightly bound. Changes in hardness, gumminess, and cohesion energy were found upon fourteen day storage and reflected amylopectin recrystallization and loss of moisture. Response of the gel microstructure to oscillatory frequency and shear was examined. It was found that solid-like properties of the confection predominated over the frequency values investigated, and the viscous modulus increased at each storage time evaluated. Similarly, shear-thinning behavior in response to increasing frequency was observed. Flow analysis indicated that the confection exhibited
Newtonian-like response to shear rates above 10 sec\(^{-1}\) and pseudoplastic behavior below the time constant. Release of bioactives reached 87% after six hours, indicating the confectionery food matrix is able to remain intact within the environment of the oral cavity and potentially be a novel delivery system for local therapeutic bioactive compounds. Comparing the release of bioactives from \textit{in vitro} methods with \textit{in vivo} testing may be difficult due to the many variables encountered in human oral cavity inter-subject variation.
3.1 Introduction to Clinical Trial

A phase I/II clinical trial was conducted at The Ohio State University to evaluate the facility of a novel starch-based confection to deliver phytochemicals from whole freeze-dried strawberries to the oral mucosa, and whether short-term consumption of the confection could provide quantifiable accumulation of strawberry phytochemicals and stimulate oral cell response in a manner that promotes oral health. A controlled crossover design was employed and comparisons between the response in healthy men and women who were active smokers or non-smokers were investigated. The specific aims of the clinical trial included:

**Specific Aim 1:** 36 men and women will be recruited and enrolled in the clinical trial, and matched based on age, gender, and smoking status. Participants will achieve excellent compliance with consumption of the strawberry gummies. Daily records of consumption will be maintained.

**Specific Aim 2:** Consumption of strawberry compared to placebo confections will result in an increase in fruit polyphenols in serum, saliva, and urine. Pharmacokinetics of strawberry polyphenols in saliva following consumption of a single dose delivering two 6
gram fruit gummies will be compared in smoking and non-smoking men and women consuming the strawberry or placebo gummies.

**Specific Aim 3:** Consumption of the strawberry gummy compared to placebo confectionery will have a greater effect on salivary inflammatory markers and modulate gene expression profiles in oral mucosa.

The focus of the present research was to complete specific aim 1 and evaluate the implementation and feasibility of the study design.

3.2 Study Design

Confectionery study agents were prepared in the Department of Food Science and Technology with formulations shown in Table 1. The six-week randomized controlled crossover study was approved by the Institutional Review Board at The Ohio State University (IRB 2010H0073) and encompassed five visits to the Ohio State University Clinical Research Center (CRC) as shown in Figure 19. Study participants were randomized to strawberry or placebo treatments. At the day -14 visit, subjects provided a paraffin-stimulated saliva sample (10 mL) followed by a saliva sample from a sterile cotton roll to evaluate baseline inflammatory markers (IgA, cortisol, 8-hydroxydeoxyguanosine), three buccal brushings using OralCDx®'s Oral Brush Biopsy (CDX Laboratories, Suffern, NY) brush tips of the right and left cheek and tongue, and two additional saliva samples collected from sterile cotton rolls for baseline saliva evaluation. At each of the five visits subjects were asked to submit a urine collection from a complete twenty-four hour period prior to their visit for quantification and
analysis of urinary polyphenols and urine cotinine, and analysis of urinary biomarkers of genetic damage and inflammation (8-hydroxydeoxyguanosine and 8-epi-prostaglandin F2α). After the pre-trial visit, participants were asked to begin an anthocyanin-free diet and take one of the provided multivitamins (CVS Pharmacy, Inc., Woonsocket, RI) daily.

After a two-week washout, subjects returned to the CRC. In addition to the biological samples previously described, fasting venous blood samples (5 mL) were drawn into two Vacutainer tubes (Becton- Dickinson, Franklin Lakes, NJ). Plasma and serum from one tube were separated from the blood cells by spinning in a centrifuge at 1000 x g (Beckman Coulter, Fullerton, CA) at 4°C. A salivary pharmacokinetic study (sPK) was also completed. For the sPK, participants were asked to tumble one dose of the study agent (two six-gram confections) in the mouth while saliva samples were collected using sterile cotton rolls at five minute intervals for thirty minutes. Block randomization to either strawberry or placebo confection treatment arms was used. Subjects were provided with seven days of the study confections and instructed to allow one dose to completely disintegrate in the mouth four times daily for seven days and record the time confection consumption started and ended in provided study diaries. Subjects’ diaries were used to determine compliance to diet, tobacco intake, and confectionery consumption. After one week of treatment, subjects returned to the CRC for a treatment visit similar to the washout visit but without the salivary pharmacokinetic study. Subjects then crossed over to the second arm of the study in which they were provided with the other type of study agent not previously consumed.
Figure 19. The use of functional confections to promote oral health in men and women controlled crossover design
3.3 Exclusion Criteria and Enrollment

Participants were recruited from flyers posted around the Ohio State University campus and smokers specifically were recruited from ResearchMatch based on proximity to campus. Smokers were matched with a non-smoker based on age and gender. Age strata were 18-34 years, 35-50 years, and 51-70 years old. Subjects were initially compensated with $50.00, but that value was increased to $100.00 to increase enrollment. Exclusion criteria did not allow individuals to participate if they had one or more metabolic or digestive illnesses, used clotting inhibitor medications, pituitary hormone disease, altered immunity, heavy alcohol consumption, dental maladies requiring treatment, oral conditions such as dry mouth, dysphagia, or salivary dysfunction, pregnancy or nursing, or were smokers who used less than ten grams of tobacco daily. Conditions of enrollment were met if subjects voluntarily agreed to participate, met exclusion requirements, did not have a body mass index of more than thirty, agreed to abstain from using mouthwashes, and agreed to complete requested study tasks including using a daily multivitamin, providing biological samples in the clinic at each of the five visits, consuming the study agent, avoid eating any foods high in anthocyanins and consuming no more than fifteen drinks of alcohol per week. The study protocol was approved by the Biomedical Sciences Institutional Review Board at The Ohio State University, Columbus, OH (2010H0073). Each subject met with a study coordinator prior to enrollment to discuss and sign the consent form and provide a Health and Lifestyle Questionnaire to determine eligibility.
After initial contact based on interest from flyers or ResearchMatch, twenty-eight potential subjects were scheduled for a pre-enrollment visit to discuss the consent form and details of participating in the study. Subjects who did not begin the study after the pre-enrollment visit were considered ineligible to participate for any of the following reasons: did not meet smoking requirement, high BMI, did not want to adhere to anthocyanin-free diet, antibiotic use within the past six months, untreated dental maladies, high alcohol intake, did not want to collect urine, loss to follow-up. Seventeen individuals were enrolled, and eleven participants began the study (Table 3).
Table 3. Subject demographics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Gender</th>
<th>Smoker</th>
<th>BMI</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>Y</td>
<td>26.9</td>
<td>Asian</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>M</td>
<td>N</td>
<td>21.7</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>M</td>
<td>Y</td>
<td>33.5</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>M</td>
<td>N</td>
<td>28.0</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>M</td>
<td>Y</td>
<td>24.6</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>M</td>
<td>Y</td>
<td>30.0</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>F</td>
<td>Y</td>
<td>29.4</td>
<td>White, Hispanic</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>F</td>
<td>N</td>
<td>32.8</td>
<td>White, Hispanic</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>M</td>
<td>Y</td>
<td>23.0</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>10</td>
<td>34</td>
<td>M</td>
<td>N</td>
<td>22.6</td>
<td>White, non-Hispanic</td>
</tr>
<tr>
<td>11</td>
<td>56</td>
<td>M</td>
<td>Y</td>
<td>31.8</td>
<td>White, non-Hispanic</td>
</tr>
</tbody>
</table>

BMI = Body mass index; Blood pressure reported as systolic / diastolic; Alcohol consumption from self-reported data indicating average drinks per day from seven day treatment period.
3.4 Study Agents and Participant Compliance

Strawberry gummies containing 48.5% freeze-dried strawberry powder were manufactured with favorable palatability. All participants completed the six-week study with one adverse event likely unrelated to the study occurred. Rapid urine cotinine analysis confirmed smoking status for all participants. Overall, participants completed the study diary and recorded the start and end time of dissolving one dose of the study agent in the mouth four times daily for seven days. One subject consumed more than fifteen alcoholic drinks during the treatment period and three subjects failed to abstain from anthocyanin-containing foods during the washout period. Four subjects failed to consume all of the total twenty-eight doses, but did not miss more than five doses. Average self-reported dissolution times of two six-gram confections during the seven day treatment period are given in Table 4. Table 5 shows the values for self-reported confection consumption times as the average of four treatments per day for the entire seven day treatment period. No identifiable trends were seen either between smokers and non-smokers or between strawberry or placebo confection consumption times. In terms of the placebo and FDS confectionery, it is important that the dissolution or consumption time is similar. However, to identify a trend between smokers and non-smokers, more participants are needed to increase the amount of data collected for analysis.
Table 4. Self-reported diet, activity level, and tobacco intake from participants in clinical trial

<table>
<thead>
<tr>
<th>Subject</th>
<th>Servings of Red and Purple Fruits or Vegetables Prior to Study</th>
<th>Activity Level(^a)</th>
<th>Daily Tobacco Intake(^b)</th>
<th>Alcohol Consumption during Treatment Period(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-7</td>
<td>Sedentary</td>
<td>8-10</td>
<td>10-15</td>
</tr>
<tr>
<td>2</td>
<td>1-3</td>
<td>Moderate</td>
<td>NS</td>
<td>1-5</td>
</tr>
<tr>
<td>3</td>
<td>4-7</td>
<td>Low</td>
<td>15*</td>
<td>3-6</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>Low</td>
<td>NS</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>Sedentary</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1-3</td>
<td>Low</td>
<td>15</td>
<td>1-5</td>
</tr>
<tr>
<td>7</td>
<td>&lt;1</td>
<td>Moderate</td>
<td>10-12</td>
<td>15-20</td>
</tr>
<tr>
<td>8</td>
<td>1-3</td>
<td>Low</td>
<td>NS</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1-3</td>
<td>Active</td>
<td>10-20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>10</td>
<td>&gt;8</td>
<td>Moderate</td>
<td>NS</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>&lt;1</td>
<td>Moderate</td>
<td>20</td>
<td>5-10</td>
</tr>
</tbody>
</table>

\(^a\)Activity levels categorized as active (>7 hours exercise per week), moderate (5-7 hours exercise per week), low (1-5 hours of exercise per week), and sedentary (no reported physical activity)

\(^b\) Represents daily cigarette intake; NS = non-smoker

\(^c\) Obtained from a study diary during the six week study.

*also reported occasional cigar usage
Table 5. Self-reported average dissolution times of strawberry and placebo confections during seven day treatment period. Times are given in minutes.

<table>
<thead>
<tr>
<th>Strawberry</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoker</td>
<td>Non-Smoker</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>8.2</td>
<td>13</td>
</tr>
<tr>
<td>59</td>
<td>8.0</td>
</tr>
<tr>
<td>8.5</td>
<td>8.0</td>
</tr>
<tr>
<td>4.9</td>
<td>5.4</td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td></td>
</tr>
</tbody>
</table>
3.5 Conclusions and Future Directions

To date, enrollment of healthy smoking and non-smoking men and women is ongoing. Eleven subjects have completed the study with no deleterious side effects; four individuals have consented to participate but are awaiting a convenient time to complete the six-week study. Initial challenges recruiting participants led to an increase in the compensation, but have not completely subsided. Numerous healthy non-smokers under 35 years old have expressed interest in participating but recruitment of healthy smokers within the same age stratum was slow. Furthermore, male smokers over 35 represent the largest percentage of participants thus far but have not been matched with healthy male non-smokers of the same age stratum due to challenges recruiting this demographic. Three of the five male smokers enrolled in the study were temporarily unemployed, which may have spurred greater interest in participating than males recruited with flyers posted around the University. Another challenge faced in recruiting smokers for the clinical trial was the need to exclude those with oral maladies or health issues, which excluded a large number of individuals interested in participating. Overall, subject compliance and adherence to the diet was good, but recruitment during the summer months dwindled as more summer fruits and berries were restricted on the anthocyanin-free diet. Issues addressed herein were considered during the program evaluation and modifications to recruitment efforts and the enrollment process were modified accordingly. To fully assess the effectiveness of the confectionery as a bioactive delivery vehicle, it is imperative to analyze the distribution of bioactives in saliva, blood, urine, and buccal tissue samples. Efforts to complete the analysis are ongoing.
Chapter 4: Conclusions

Strawberry phytochemicals have been shown to elicit significant protective effects on oral mucosa when evaluated in pre-clinical animal models. Specifically, phytochemicals with demonstrated free radical scavenging, inflammatory response-mediating and chemopreventive activity are of interest. When in contact with oral mucosa for a therapeutically-effective period, the known health-promoting compounds in strawberries have the potential to be ideal disease preventing agents. Providing health-conscious consumers with a functional confectionery product that can confer the natural protective effects of strawberry phytochemicals is a unique approach to promoting oral health and overall well-being. Although traditional pharmaceutical forms can be effective vehicles of phytochemical delivery, incorporating whole freeze-dried strawberries may be a challenge. A novel starch-based confection that can be conveniently consumed without mastication may be desirable to not only healthy individuals, but also to those with difficulty eating due to loss of teeth, oral sores, and other oral maladies. An investigation into the physical properties that allow a confectionery product to function as a fruit bioactive delivery system was described herein.

Optimization of a formulation for a starch-based confectionery product containing 48.5% w/w whole freeze-dried strawberry powder was achieved. Characterization of physical
properties related to oral processing and shelf-stability was completed. Thermogravimetric analysis revealed the FDS confectionery form contained 22.3% moisture, which decreased upon fourteen-day storage to 18.2%. Loss of moisture upon heating occurred at higher temperatures, indicating water was more tightly bound to the macromolecular components of the confectionery system. The decrease in water during storage manifested as a significant increase in hardness, chewiness, and gumminess as determined by texture profile analysis. Providing a delivery vehicle that is durable enough to withstand oral manipulation is crucial for effective bioactive deposition within the mouth. Increasing the force needed to manipulate the confection for bolus formation may be beneficial. It was determined with small amplitude oscillatory testing that the confectionery matrix had predominately solid-like properties, indicating that upon small deformation energy is not lost and the system can recover. This, too, is ideal for a delivery system that will undergo changes in shear within the oral cavity. Additionally, temperature-dependent changes were found to be minimal and it can be assumed that the temperature differences upon consumption will not impede the functionality of the confection. Release of polyphenols and monomeric anthocyanins was evaluated by dissolution testing in simulated saliva, and showed nearly 90% release after six hours. Slow release of bioactives is ideal, as constant washing by saliva and swallowing can significantly reduce the dose delivered to the target oral tissue. To evaluate the facility of the confectionery as a strawberry bioactive delivery vehicle, it was necessary to conduct a clinical study.
The pilot clinical trial outlined herein was designed to investigate whether short-term administration of a whole food product such as strawberries delivered as a gummy confection resulted in measurable phytonutrient deposition within the oral mucosa, and corresponding modulation of a series of genes known to be associated with oxidation and inflammation without inducing significant adverse effects. The phase I/II placebo controlled crossover design involved two groups of male and female healthy participants (smokers and non-smokers) where a confection containing strawberry fruit was evaluated. Using flyers and ResearchMatch.com, forty individuals were recruited and eleven participants completed the six-week clinical trial without any reported toxic or adverse events due to the study agent. Compliance to the diet and consuming the study agent was good. Efforts to continue enrollment and program evaluation are on-going.

In conclusion, it was found that the physicochemical characterization of a novel fruit confectionery revealed potential for use as a bioactive delivery form. A phase I/II clinical trial was conducted to confirm the prospective use for the prevention of oral maladies.
References


Chen, J. (2009). Food oral processing—A review. Food Hydrocolloids, 23(1)1-25


Is there a place for healthiness in the indulgent world of candy, chocolate and gum? *Nutraceuticals World*.


Appendix A. Eligibility Checklist for Clinical Trial
# Strawberry Gummy Screening Checklist

**Screen Date:**

**Name:**

**DOB:**

**Male [ ] Female [ ]**

**Nationality / Race:**

**Occupation:**

**Home address:**

**Phone No. (H) [ ] (C) [ ]**

**Email:**

**Best time to call:**

**Which is the best way to contact you email and/or phone:**

---

<table>
<thead>
<tr>
<th>Subject is NOT eligible if any of the responses are marked as NO</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Subject has no allergy to strawberries, wheat, corn, or products made with these foods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. This subject is a smoker who smokes more than 10 cigarettes or equivalent to 10 grams of tobacco daily and will continue to do so while on this study OR This subject is a non-smoker who has either never smoked or has quit smoking for more than 10 years and not more than 1 pack/day. This subject will continue to not smoke while on this study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Subject is between the ages of 18 to 70 years old [ ] Premenopausal women [ ] Postmenopausal women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Subject agrees to consume a standardized vitamin &amp; mineral supplement regime and avoid all other dietary supplements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Subject agrees to follow anthocyanin-free diet which means to avoid all foods described in strawberry diary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Subject agrees to avoid the use of any mouthwashes throughout the study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Subject has no history of malabsorptive, GI, metabolic disorders, cavities, oral lesions, loss of significant number of GI organs, anemia, hemophilia, on warfarin sulfate, or have problems with blood clotting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Subject has no difficulty or pain with swallowing or does not have chronic dry mouth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Subject does not consume ≥ 15 servings of alcohol / week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Subject has not been on antibiotics in the last 6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Subject does not plan on becoming pregnant while on study or is currently nursing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix B. Health and Lifestyle Questionnaire
HEALTH & LIFESTYLE QUESTIONNAIRE

Date________________

Name_________________  Birth date_________  Male ☐  Female ☐

Subject Information
Nationality / Race: ________________  Occupation: ________________
Home address: ________________  Phone No. (H) ________________
                   ________________ (W) ________________
                   ________________ (C) ________________
Email: ________________
Which is the best way to contact you email and/ or phone? ________________
best time to call? ________________

Diet / Exercise
1. Do you have any allergies (food or medicines)? Yes ☐  No ☐
   If yes, please specify your allergy and the extent of your allergic reaction:
   ____________________________________________________________________

2. Have you ever eaten foods containing strawberries, corn, or wheat?
   Yes ☐  No ☐

3. In your regular diet, do you frequently consume red and purple colored fruits or vegetables?
   Yes ☐  No ☐
   If so, how many servings a week?
   <1 ☐  1 to 3 ☐  4 to 7 ☐  >8 ☐

4. Do you follow a particular diet? Yes ☐  No ☐
   If yes, please check all that apply.
   ____________________________________________________________________
   low fat diet ☐  high fiber diet ☐
   low carbohydrate diet ☐  no dairy (lactose free) ☐
   Atkins diet ☐  macrobiotic ☐
   The Zone diet ☐  low sugar diet ☐
   40/30/30 diet ☐  low sodium diet ☐
   _______ (other) ☐  high calorie diet (weight gain) ☐
   diabetic diet ☐  low calorie diet (weight loss) ☐
   vegetarian diet ☐  other__________ ☐
5. Do you exercise regularly?  
If yes, please describe your weekly routine (please approximate time spent in each activity)  
__________________________________________________________________________

Medical History  

For male participants please skip to question 4. The first three questions are for female participants.  

1. Do you know or suspect that you are currently pregnant, are lactating, or plan to be pregnant during this 9-week study?  
Yes □ No □

2. Are you currently taking any oral contraceptives? Yes □ No □

3. Are you menopausal? Yes □ No □  
When was your last menses? _______ Date

4. Are you lactose intolerant? Yes □ No □  
If yes, how many servings of milk or dairy based products can you tolerate a day?  
0 servings □ 1 serving □ 2 servings □
Do you use lactase (Lactaid®)? Yes □ No □

5. Have you taken antibiotics in the last 6 months? Yes □ No □  
If yes, when____________ how long __________ reason ______________

6. Have you ever had or currently have any medical problems with the following? If yes, check box  

Blood (i.e. anemia, bleeding) □  Joints (i.e. arthritis) □
Chronic Illness (i.e. diabetes) □  Kidney □
Eating disorders (i.e. bulimia, anorexia) □  Large intestine □
Swallowing (dysphagia) □  Liver (i.e. hepatitis) □
Gall Bladder □  Small intestine □
Immune (i.e. lupus, cancer) □  Thyroid or Pituitary □

*If you have checked any of the above boxes, please describe the medical problem.
__________________________________________________________________________

7. Have you ever had surgery on any of the following organs? If yes, check box
Stomach  Intestines  Thyroid
Gall Bladder  Liver  Pituitary

8. Do you take prescription medications?
   Please list with dose taken each day (if known).

   ___________________________  ___________________________

   ___________________________  ___________________________

   ___________________________  ___________________________

   ___________________________  ___________________________

9. Do you take vitamin or mineral supplements or dietary supplements?
   Please list with dose taken each day (if known).

   ___________________________

   ___________________________

   ___________________________

   ___________________________

10. Do you take any herbal, botanical or “alternative-medicine” preparations?
    Please list with dose taken each day (if known).

    ___________________________

    ___________________________

    ___________________________

    ___________________________

Dental/Oral History

1. Have you had any oral surgery in the last 3 months?
   Yes □  No □
   If yes, where  ____________  when  ____________

2. Have you ever been told that you have periodontal disease (periodontitis)?
   Yes □  No □
   If yes, when  ____________  Type/severity  ____________
   Did you need treatment for periodontitis?
3. Do you have any open sores or had any open sores in the last month?
   Yes ☐ No ☐
   If yes, where __________ when __________

4. Do you have any cavities or cracked teeth that have not been treated?
   Yes ☐ No ☐

5. Are you currently using any prescribed or medicated mouth rinses?
   Yes ☐ No ☐
   If yes, name __________ reason __________

**Smoking History**

1. Do you currently smoke?
   Yes ☐ No ☐
   If YES, how many of the following: cigarettes per day? ______
   *cigars per day? __________ *pipe use per day? __________
   *Amount of tobacco can vary in these products therefore please provide as much detail about the size (small, medium, large cigar) or the amount of pipe tobacco used (1 teaspoon or tablespoon).

   If NO, have you smoked in the past? Yes ☐ No ☐ (go to question 3)
   If YES, you have smoked in the past
   a. How long has it been since your last cigarette: _____years _____months
   b. What was your daily cigarette use? _____packs per day
   c. How long did you smoke? _____years _____months

2. Are you a social or binge smoker? Yes ☐ No ☐

3. If you have never smoked in the past have you currently or in the past used any of the following products:
   Chewing tobacco ☐ Dipping tobaccos ☐ Snuff ☐
   Creamy snuff ☐ Hookahs/ shishas ☐
   Other* __________ ☐
   If yes, for how long _____years _____months
   How frequent? Daily ☐ 3x a week ☐ once a week ☐
4. Do you live with a smoker?   Yes ☐   No ☐

**Alcohol Consumption**

1. Do you drink alcohol?
   Yes ☐   No ☐

2. How many alcoholic drinks* do you regularly drink in one week?   ______
   *one drink = 1.5 ounce liquor, 6 ounces wine, or 12 ounces beer

3. Do you use mouth washes containing alcohol (Listerine)?
   Yes ☐   No ☐

   If yes, how many times a day?
   once ☐   twice ☐   more than 3 times ☐

   For how long?   ______ years   _____ months
Appendix C. Participant Diary Entry for Self-Reported Confection Consumption
Name:__________________   Day: _____________  Date: _____________  Week: ___

Have you smoked in the last 24 hours?  □No     □Yes

If yes, what was smoked? __________

How many? □less than 5  □6 to 10   □10 to 15   □more than 16

How many alcoholic beverages have you consumed in the last 24 hours?
□None    □less than 2   □2 to 5    □6 to 10   □more than 10

Instructions: Place an “X” in the box when you have consumed each piece of strawberry confection (gummy). Please complete one of these forms every day until the end of the study.

<table>
<thead>
<tr>
<th>PIECE 1</th>
<th>PIECE 2</th>
<th>Time Started</th>
<th>Time Finished</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gummy 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gummy 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gummy 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gummy 4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional Anthocyanin Food: If you accidentally consumed any of the foods on the “Do Not Eat” list, please list the food and the amount consumed in the space below.

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Quantity</th>
<th>Food Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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</tbody>
</table>