THE EFFECTS OF SOY PROTEIN ISOLATE ADDITION ON THE PHYSICO-CHEMICAL PROPERTIES OF GUMMI CONFECTIONS

THESIS

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ABSTRACT

Soy protein is commonly used to enhance the nutritional quality of food. However, it also has the ability to act as a functional ingredient, imparting unique characteristics to foods to which it is added. The effects of soy protein isolate incorporation on the physico-chemical properties of starch-based gummi confections were investigated using thermal and rheological analyses, as well as supportive sensory and spectroscopy studies. The overall objectives of this study were to first characterize the textural and rheological changes upon varying levels of soy addition to starch confections and consequent changes to quality parameters and second to assess the impact of incorporation of soy protein isolate on storage stability.

Texture profile analysis was conducted to simulate mastication and to quantify, along with a hedonic sensory panel, the effects on gummi acceptability as a function of texture. Increasing levels of soy protein yielded samples that were progressively less firm and cohesive, caused either by starch network dilution and/or disruption. The softening effect of soy protein was observed throughout storage. Addition of soy protein improved the texture acceptability, presumably by decreasing perceived shortness, a texture defect associated with overly-firm gelled confections. Rheometric analyses were conducted to determine the relationship between gummi viscoelastic properties and the applied stress and rate thereof. Addition of soy protein was found to progressively
suppress the yield increase at each storage interval. The viscoelastic crossover frequency, an indicator of viscoelastic stability, was also found to decrease as soy protein concentration increased and to have a lower rate of increase during storage.

Additionally, water-dependent interactions were characterized as they relate to soy concentration and storage time. Thermogravimetric analysis results indicated that the primary water population was more easily removed in high-soy protein formulations. Additionally, as storage time increased, soy protein maintained relative homogeneity of the primary water population and removal of water required lower temperatures with the soy formulation, indicating less entrapment of water throughout the storage period studied.

Results from differential scanning calorimetry did not indicate a significant shift in the glass transition temperature as soy concentration increased, most likely due to the low concentration of plasticizing water. Neither gummi system exhibited distinct endotherms associated with starch melting regardless of treatment; soy protein inclusion did not prevent complete starch gelatinization during the confection process, as evidenced by the lack of the typical starch melting endotherm. However, over time, the addition of soy protein was able to prevent an increase in the $T_g$ of the gummi confections, perhaps by interfering with starch re-association and retrogradation.

The changes in monomeric anthocyanin quantity, expressed as units of cyanidin 3-glucoside, which occurred as a result of confection processing were also analyzed using ultraviolet spectroscopy. The soy gummi confections yielded higher anthocyanin recovery compared to the standard formulation. This is probably due to the greater heat
dissipation caused by a lower cooking viscosity from the soy formulation during the confection process. Sensory analysis, conducted using a hedonic scale of acceptability, showed that addition of soy protein improved acceptance for both texture and flavor. The mechanism of improvement is thought to be related to the decreased rigidity (shortness) of the product which augments mouth-feel and possibly flavor release.

All results indicate that soy protein has potential as a processing aide in gelled confections by modulating physical properties. Gummi gel rheology was shown to be dependent on soy concentration and storage time. Texture became less firm with increasing soy protein concentration, and soy protein was shown to decrease firming over time. The physical results support those of sensory analysis, in which it was found that soy protein improved acceptability of the gummi confections for both texture and flavor. The distribution of water and its dynamics were also shown to be time and concentration dependent. Finally, addition of soy protein may aide in the processing stability of anthocyanins by increasing heat dissipation.
DEDICATION

This document is dedicated to my grandmother Stella
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FIELDS OF STUDY

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CHAPTER 1

INTRODUCTION

Gelled confections and candies, collectively referred to as gummies (Edwards, 2000), are a large, stable, and growing business (Young, 1998); As of 2006, industry experts have reported as high as 8.4% growth in the gummi candy sector, mostly attributed to innovative formulations (Anon, 2006). Many of the most common and recognizable gummi confections are typically made with gelatin as the active agent of gelation (Lennox 2002). However, gelatin is not the only option for creating gummi-type candies. Starches may be preferred for being free of animal by-products and the associated concerns (Lennox 2002). However, starch-based confections are sometimes described as “short” an undesirable texture attribute characterized by a firm, brittle gel (Burg, 1998).

Texture is a primary determinant of quality in gelled confectionery products and, due to their relatively simple composition, provide an ideal model for composite gel investigations (DeMars & Ziegler, 2001). Gumminess and chewiness are texture analysis descriptors that are particularly applicable to gelled confections (Borwankar, 1992). Firmer (short) gummies that are more prone to fracture during mastication are often described as chewy and softer gummies that dissolve during mastication are described as gummy (DeMars & Ziegler, 2001). One of the primary uses of soy protein...
in foods is as a texture modifier (Lusas & Riaz, 1995). Depending on the polysaccharide gel system, soy protein has been shown to have variable results, enhancing (Baeza et al, 2002) or disrupting the gel's structural integrity (Ryan & Brewer, 2005). Addition of soy protein ingredients to confections was suggested to improve handling properties and stickiness (Rhee, 1994). Therefore, in gelled starch-sugar confections, a relatively model gel system, soy proteins would perhaps be beneficial. Therefore, our hypothesis was that soy protein affects the texture of the starch-based confections on a concentration-dependent basis. It is also hypothesized that soy protein will have a lasting effect on the texture and related properties of starch-based confections throughout the defined storage period.

To understand the textural changes that were affected upon addition of soy protein, the rheological behavior was assessed. Oscillatory stress sweeps (OSS) reveal the viscoelastic properties and behaviors of foods. Soy protein has been shown to have a pronounced effect on viscoelasticity in gel and food systems (Ryan & Brewer, 2005; Vittadini & Vodovotz, 2003). Oscillating frequency sweeps (OFS) were used to characterize the rheological properties of the confections as a function of time. In these gelled systems, OFS can reveal long-term stability (Rao, 2007), such as tendencies for phase separation, retrogradation, syneresis, or sedimentation. If the addition of soy protein creates a systemic instability, the oscillatory stress and frequency sweeps should reveal differences between the various concentration treatments and time intervals.

In addition to the solid components of the gummi confection, water is another critical component that affects the overall physical properties. Traditional methods of describing water behavior (water activity, moisture content, and solvent retention
capacity) do not adequately describe the distribution and mobility of water in food systems (Slade et al., 1991). Thermogravimetric analysis (TGA) can be used to characterize the changes in physico-chemical properties of a food system as a function of temperature. TGA can be used to track rate of moisture loss and differentiate different water populations within a multi-component food (Fessas and Schiraldi, 2000). Therefore, TGA will be used to characterize and compare water populations in the gummi confection system and how those are affected by addition of soy protein isolate.

The aim of this investigation was to assess the effect of soy protein addition on the physico-chemical properties of a starch confection system. Specifically, the textural, thermal, and rheological properties of starch-based grape juice confections will be evaluated upon incorporation of multiple levels of soy protein. The effect of soy protein on monomeric anthocyanin stability, such as cyanidin-3-glucoside (see Appendix A), which is known to be compromised in high-temperature food preparation methods (Patras et al., 2010; Edwards, 2000), will also be assessed in the gummi confections. The sensory acceptability of gummi confections made with soy protein will be evaluated by an untrained panel with particular emphasis on texture and flavor. Lastly, the storage stability of gummi confections formulated with and without soy protein will be assessed through 10 and 20 days using thermal and rheological characterizations.
CHAPTER 2

STATEMENT OF PROBLEM

In addition to its recognized nutritional value, soy protein has demonstrated potential in food systems as a functional ingredient. Its use in other food systems has demonstrated a potent capacity to change water dynamics and rheological properties; therefore, it may have potential as a texture enhancer. It is possible that formulating gummi confections with soy protein may improve texture and stability.

How soy protein addition will affect the physico-chemical properties and processing of confectionery products has not been formally investigated. It is therefore vital that an understanding of component interactions be attained in order to successfully produce acceptable soy-formulated foods. The objective of this investigation was to characterize the effects of soy protein isolate on the physico-chemical properties of a starch-set, gummi-type confection as a function of storage time and soy protein content.

Hypothesis: Soy protein concentration and storage time will modulate the physico-chemical properties of starch-based gummi confections by changing the physical nature of the gel matrix and water distribution. These changes are proposed to be caused by altering the interactions between system components (water, sugars, starches, and proteins). The following aims are proposed to investigate this hypothesis:
**Aim 1:** To characterize and compare the thermal and rheological properties of starch-based gummies with multiple levels of soy protein incorporation.

**Aim 2:** To characterize the changes that occur in gummi candies with and without soy protein during storage.

**Aim 3:** To determine how soy protein addition affects the sensory acceptability and processing stability of anthocyanins using UV spectroscopy.
LITERATURE REVIEW

3.1 Gummi Confections

In 2009, non-chocolate chewy confectionery sales increased by 11.2% (Anon, 2010). Chewy sugar-based candies are a stable part of the American confectionery industry (Young, 1998) with sales of over $1.6 billion (Anon, 2010). However, the soft candy sector of the sugar confectionery industry had recently suffered losses to production and value (USCB 2005) that industry professionals have attributed to lack of innovative products (Anon, 2006b). Development of a highly acceptable soy-containing gummi confection may represent a key new niche for this industry and a passageway into the area of functional foods.

3.2 Soy functionality in foods

The use of soy protein in foods for nutritional purposes is an established area of food augmentation (Green et al., 2006; Sethi et al., 2007; Prasad, 2009; Serventi et al., 2009) that is becoming more and more accepted by Americans (Nelson, 2008). However, it also has great potential as a functional ingredient, imparting unique physical properties when incorporated into the formulation (Vittadini & Vodovotz, 2003; Lusaz & Riaz, 1995). Soy protein has also been implicated improving product stability, such as impeding product staling (Vittadini & Vodovotz, 2003; Anhong et al., 2006) and freeze-
thaw stability (Akesowan & Taweesakulvatchara, 2009), perhaps by simple moisture retention or by interacting with amylopectin and retarding retrogradation (Sciarini et al., 2008). However, the use of soy products in confections is limited and is usually restricted to a supporting role for other ingredients (Endres et al., 2003) due to a variety of reasons. For example, adverse texture and sensory attributes (chalky, floury, beany, etc.) of soy limit its application (Starling, 2005). However, addition of soy protein ingredients to confections has been suggested to improve handling properties and stickiness (Rhee, 1994). Soy protein requires more energy and time to completely disperse and hydrate (Mai, 2004). Maillard browning reactions may also compromise visual appeal in soy protein fortified confections if the system is rich in reducing sugars and not sufficiently acidic.

3.3 The role of ingredients in gummy confections

Confectionery gels typically consist of sugars, water, and a gelling agent (Burey et al., 2009). The choice of gelling agent can have the biggest impact on final product quality and attributes, and therefore must receive special attention. Gelatin, the traditional gelling agent used in gummy formulations, is losing favor due to cultural, dietary, and safety concerns (Lennox, 2002; McHugh, 2003), as well as the desire for unique textural properties (Poppe, 1995). Specialty starches have been developed to meet the demand for gelatin-free candies while providing unique sensory and physical properties (Warnecke, 1991). Starch-set gummies, the largest volume gum/jelly product in the United States (Warnecke, 1991), have greater acid and heat stability and have much shorter gelation time than gelatin gummies (Burg, 1998), thereby conferring functional and pecuniary advantages. The most commonly used starches for gummi and
soft jelly applications are acid-thinned because they have relatively low hot viscosity (Ellis et al., 1998; Singh et al., 2007) and can therefore be agitated and pumped with minimum energy expenditure. Wheat starches have received renewed attention due to their unique properties (Sloan, 2006). Gummies are traditionally made in a batch process (Scuderi, 2002; Burey et al., 2009) which, although not modern, does prevent mechanical damage upon the swollen starch granule (Zallie, 2006). In a standard starch-based soft candy, a thin boiling starch is heated in water beyond the gelatinization temperature, mixed with a corn syrup – sucrose solution, and boiled down to the desired soluble solids content. In contrast to relatively well-understood gelatin gummy formulations, the interactions that occur between water and starch in a high sugar environment (>65%, such as in gummy-like confections) have not been studied adequately. Chang et al., (2002) demonstrated that certain sugars can, in fact, plasticize starch and reduce firmness, but at lower concentrations. Kasapsis et al., (2004) showed that high sugar concentrations reduce the structural order of a model confection system, as opposed to gelatin which is reinforced by high sugar (Kasapsis et al., 2003).

Composite or mixed gels are becoming more common in foods due to the desire for unique texture and handling characteristics (DeMars & Ziegler, 2001; Burey et al., 2009). However, the interactions (or lacks thereof) that create such a novel system are less well understood. In protein-polysaccharide systems, the level of interaction or separation depends on many factors, such as system pH, moisture, material history, as well as polymer properties such as charge, size, and morphology (Burey et al., 2009). For gelled confectionery systems, which are held together by a continuous network whose dynamics are retarded by high solutes, an added protein may create a
discontinuous phase if it is non-structure forming and competes for limited solvent (Burey et al., 2009) which could theoretically render the entire system discontinuous at some critical concentration.

The sweetness in gummi and similar confections is achieved primarily by high fructose corn syrup (HFCS), which is functionally preferable over pure sucrose due to the crystallization-inhibiting abilities of fructose; crystallization being an undesirable defect in soft, sugar confections (Warnecke, 1991). Similar sweetness and functionality can be realized by using fruit juice concentrate with the added advantage of naturally contributing pigments (such as anthocyanins), which may impart additional health benefits (Prior et al., 2000). Concord grape juice (CGJ) flavonoids are potent antioxidants that may protect against oxidative stress and reduce the risk of free radical damage and chronic diseases (O’Byrne et al., 2002). Grape anthocyanins are over 50% acylated (Tamborra et al., 2006) and are therefore considered to be more bio-available (Harada et al., 2004) and their deep purple color is less likely to fade over time (Bassa et al., 1987). Additionally, concentrating fruit juice further promotes product color stability (Giusti et al., 2003). Lastly, the low pH of CGJ (2.8) may help inhibit Maillard browning reactions. Anthocyanin content is often expressed as units of cyanidin-3-glucose (Miyazawa et al., 1999; structure displayed in Appendix A).

3.4 The role of water in gummy confections

Starch-protein interactions and the behavior of water in a high-solids’ environment, like confections, as they pertain to product stability have not been investigated. Gel-formation capacity and viscosity of soy protein hydrates is related to soy protein composition (native vs. denatured and the extent of denaturation),
temperature, and pH (Arrese et al., 1991). Ideally, soy protein and starch will each contribute to gel formation and firmness. However, soy protein isolate (SPI) may affect sugar recrystallization, glass transitions, starch retrogradation, and other physical effects of soft jelly candy as observed in other food systems (Vittadini et al., 2003; Luo et al., 2003; Tsai et al., 1998). Therefore, to understand how SPI affects the thermal and physical properties of starch-based soft jelly confections, it is important to first characterize the physico-chemical properties of the model system without SPI incorporation.

A basic soft jelly and gummy formulation is a heterogeneous mixture of water, sugars, and gel-forming polymers. In such a system, water acts as a plasticizer and lowers the glass transition temperature \( T_g \) of food constituents. Fructose has also demonstrated high plasticization capacity on the amylopectin fraction of starch (Liu et al., 2004). In boiled sweets, like gummy candies and toffees in which final water content is low (<20%), the product is in an amorphous rubber (preferable) or an amorphous glass, depending on the extent and rate of water removal (Roos et al., 1991). The addition of polymeric compounds, such as starch and protein, to a sugar solution tends to increase the \( T_g \) (Rogers et al., 2006), slowing crystallization of amorphous sugars due to decrease free volume, molecular mobility, and diffusivity (Roos et al., 1996).

Changes in water mobility, independent of moisture content, may be caused by continuous chemical interactions in the system, such as recrystallization, starch retrogradation, and glassy/rubbery equilibrium (Cornillon et al., 2000) affecting, among other things, the shelf life of the product. For example, recrystallization of amorphous sugars due to increased molecular mobility is an undesirable defect in soft, gummy-like
candies because it creates a coarse texture (Hartel, 1993). Additionally, in a system where anthocyanins are present, low water mobility results in greater stability of the pigments (Tsai et al., 2004). Water may also exist in multiple populations because of the heterogeneity of the system; to what extent water is associated with gelatinized starch and how that may change upon soy addition in a low-water, high-sugar environment is also unknown. Characterizing the behavior of water is therefore essential to understanding the molecular dynamics and estimating stability of gummy-like candies.

Established methods of examining water behavior (water activity, moisture content, and solvent retention capacity) do not adequately describe the distribution and mobility of water in food systems (Slade et al., 1991). Thermal analytical techniques, including differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), can be used to characterize the changes in physico-chemical properties of a food system as a function of temperature.

The DSC detects phase changes indirectly by comparing the sample heat flow rate to an inert control as both are exposed to the same change in temperature (Schenz, 2003). What is directly measured is the amount of heat required to keep the sample cell at the same temperature as the reference, which is commonly an empty pan (Schenz, 2003). This heat amount will increase in an endothermic reaction such as melting or decrease in an exothermic reaction such as crystallization. Melting and crystallization are both 1st order transitions, so upon reaching the melting temperature \( T_m \), a sample’s temperature will not rise until all the crystals have melted, meaning even more heat must be applied to keep the temperature rising at a constant rate relative to the reference cell (Lind, 1991). Most foods are a mixture of solids, liquids, and amorphous materials, which presents
challenges when attempting to characterize individual transitions. Some first order transitions, such as starch gelatinization, amylopectin re-crystallization, and “freezable” water melting have all been quantified using DSC in select carbohydrate systems (Takashi & Yamada, 1998; Vittadini & Vodovotz, 2003).

Additionally, DSC can be used to quantify 2\textsuperscript{nd} order transitions, such as the glassy-rubbery transition. This unique transition is associated with an increase in molecular motion of the amorphous phase of polymers as they change from the glassy (rigid) to the rubbery (flexible) state (Slade et al., 1991; Walstra, 2008). This reversible change occurs at a specific temperature known as the glass transition temperature ($T_g$). The glass-to-rubber transition is a 2\textsuperscript{nd} order endothermic reaction, meaning that while heat is required to complete the transition, latent heat is not a factor (Wunderlich, 1994). Glass transitions are observed under DSC analysis as a sigmoidal change in the heat capacity of a sample (Wunderlich, 1994). If the $T_g$ of the system or ingredient is above a given temperature ($t_o$), the system is in a glassy state (rigid) at $t_o$ with limited polymeric motion in the amorphous phase. Conversely, if the $T_g$ of the system or ingredient is below $t_o$, the system is in a rubbery (flexible) state and the polymeric mobility is high. Figure 3.1 below depicts a typical DSC thermogram of a starch-based gummi confection.
Figure 1  Typical DSC thermogram of a gummi confection made with wheat starch. The highlighted area is a putative glass transition.
Thermogravimetric analysis (TGA) is a thermo-analytical technique used to determine the distribution of water within a heterogeneous system by monitoring weight loss of a material as a function of time or temperature. Samples may either be held at a constant temperature to examine time-dependent changes, such as oxidation and evaporation, or be subjected to linear or step-wise temperature increases to determine temperature dependent reactions (Fessas & Schiraldi, 2001). Derivative weight loss curves provide a peak that distinctly plots the temperature range(s) of greatest rate of weight loss (Figure 3.2). In food systems, this is usually weight loss attributed to water.

Experimental conditions and sample treatment history must be tightly controlled for reproducibility. To conduct TGA experiments, small samples representative of the whole food are placed into an appropriate, non-reactive sample pan. This pan is attached to one end of a balance, the other end holding an empty pan. The sample is then loaded into a furnace and subjected to the time or temperature treatment. During the experiment, the interior of the furnace is continuously purged with gas (or a controlled series of gasses) that may be either non-reactive or reactive with the sample, depending on the aim of the experiment. Data acquired from TGA experiments are usually plotted as weight or %weight versus time or temperature. While these can themselves be used for determining the amount of a certain component within the sample, such as for proximate analyses, little is revealed about rates of weight change. Applying the first derivatives of these curves reveal these rates of weight loss and can more accurately describe composition of the sample and can also reveal the most stable (little or no weight change) regions of time or temperature.
Fessas and Schiraldi (2001) have described the use of TGA in bakery products to monitor the rate of water release as a function of temperature, assigning ingredient effects to the different components of the food. This means that water is not uniformly partitioned within foods. Water that is less strongly associated with food components is removed at lower temperatures by a simple diffusion mechanism (Fessas & Schiraldi, 2001), whereas strongly associated water is removed at high temperatures by a change in the physical properties of the system itself. Therefore, using TGA will characterize the water distribution of a standard starch-based soft jelly candy and the changes arising from the addition of SPI to the candy formulation.
Figure 2  Typical TGA thermogram of wheat starch-based gummi confections. Events at temperatures greater than 160 oC are considered sample destruction.
3.5 Characterization of physical properties

Texture is a primary determinant of quality in confectionery products and, due to their relatively simple composition, provide an ideal model for texture-structure investigations (DeMars & Ziegler, 1998). The Instron Universal Testing Machine can be used to measure the effects of large deformations on a material and is ideal for gelled sugar confections. When correlated with sensory analysis of foods, texture analysis may be used as a quantitative supplement to sensory panels in the evaluation of texture (Szczesniak, 1986). Texture profiles analysis (TPA) is a common and robust method to analyze foods in a way meant to compare to mastication (Daubert & Foegeding, 2003). From a TPA (see example in Figure 3.3), it is possible to determine various physical properties of the material. Hardness (resistance to deformation) is expressed as the maximum force applied during the first compression and is usually, but not always, the maximum compression point. Cohesiveness, a measure of the intermolecular forces within a material, is measured as a relative resistance to deformation between the two compressions. Lastly, gumminess is a product of the hardness and the cohesiveness and is defined as the force required to masticate the material to dissolution (Szczesniak, 1962). Using a mixed component gummi confection, DeMars & Zielgler (2001) correlated analysis of texture rigidity with sensory analysis of perceived mouth-feel, and even observed that it impacted flavor perception. Therefore, texture profile analysis can be used to characterize shortness, a texture defect in gummi confections characterized by firm, brittle gels (Burg, 1998), the effects it has on sensory acceptability, and how soy protein addition impacts shortness perception.
Figure 3  Typical texture profile analysis for starch-based gummi confections.
By definition, rheology is the study of deformation and flow (Rao, 1999), which would therefore include texture analysis. However, the underlying viscoelastic properties that manifest as textural changes can be probed by small-deformation rheological analysis. Using a rheometer, it is possible to observe the viscoelastic behavior of a given material as a function of applied stress and the rate thereof. Oscillatory rheometry is an established technique for measuring flow and deformation of food matter under applied stress or strain (Rao, 2007). Rheological properties are also likely to correlate strongly with mouth-feel (Sukha et al., 2003). Oscillatory stress sweeps can reveal basic relationships between a food's elastic and viscous behavior. When $G' > G''$, the sample behaves more like an elastic solid and has higher stress resistance (Brummer, 2005). Yield stress, or the stress at which a material is strained beyond its linear viscoelastic region (LVR) and begins to deform permanently (Rao, 2007), is indicative of a gel's structural integrity. Figure 3.4 depicts the results of a typical yield stress analysis using an oscillatory stress sweep.
Figure 3.4. Example of the yield stress (seen as the onset point of decay) of a material being subjected to an increasing oscillatory stress sweep.

Figure 4. Example of the yield stress (seen as the onset point of decay) of a material being subjected to an increasing oscillatory stress sweep.

Onset Point
osc stress = 171.3 Pa
G' = 2042 Pa
In addition to stress sweeps, frequency sweeps can also reveal dependency on rate of applied stress and how that relates to micro-structural stability (Pai & Khan, 2002). In particular, the viscoelastic crossover (G’-G’’) cross-over frequency can be predictive of long-term stability (Ross-Murphy, 1994) and any interactions which may affect it (Franco et al., 1997). For example, a liquid food material displaying a frequency-independent elastic modulus (G’) with a steadily decreasing viscous modulus (G’’) at high frequencies may be experiencing extensive polymeric interactions and behave more like a solid (Ikeda & Nishinari, 2001; Sans et al., 2005), something with serious implications for an industrial food manufacturer. Alternately, high frequencies can induce polymer dissocation and cause a gel material to behave more like a liquid (Gigli et al., 2009).

Figure 3.5 depicts the cross-over frequency of a typical oscillatory frequency sweep analysis on a viscoelastic gel (frequency dependency presents itself as changing slopes on the log-log axes). Sivaramakrishnan et al., 2004, used an oscillatory frequency sweep to characterize how an added hydrocolloid can disrupt the normal structural associations within a model bread system and at what point the dominant structural component is the added ingredient.
Figure 5  Viscoelastic crossover (G'-G") frequency obtained from an oscillatory frequency sweep.
CHAPTER 4

METHODOLOGY

4.1 Gummi Formulation and Manufacture

Confections were prepared using the ingredients listed in Table 1 as follows: all ingredients were mixed by shaking prior to pouring into the kettle (30 qt., jacketed, plus scraping mixer; Schweppes, Addison, IL 60101). Water and 65 °Brix concord grape juice concentrate (Welch's Foods Inc., Concord, MA 01742) amounts were equivalent across all variables. Acid-thinned wheat starch (Gemstar 1090, Manildra Group USA, Shawnee Mission, KS 66205) and soy protein isolate (Prolisse, Cargill, Inc., Minnetonka, MN 55391) were added to the juice-water solution and then heated to cooking temperature, which was set to and maintained at 100 °C. Confections were subsequently cooked to a final soluble solids content of 70 °Brix (approximately 25 minutes), verified using a high-solids refractometer (Fisher Scientific, Pittsburgh, PA 15275). Confections were then set in Clean Set™ 0736 cornstarch (Cargill Inc., Cedar Rapids, IA 52406) and cured/stoved for 24 hours at 38 °C in an Isotemp® drying oven (200 series, model 215F, Fisher Scientific, Pittsburgh, PA 15275). The final total solids content was approximately 78-80 °Brix for all formulations, where 80 °Brix is considered standard for gummi-type confections (Koh & Mulvaney, 2004). All equipment used in the gummi batching process is shown in Figure 6. After curing, candies were cooled to room temperature, gently brushed free of cornstarch, and then heat-sealed in polyethylene bags.
Samples were stored at room temperature (~ 20 °C) until analysis at day 1, 10, and 20 (note: concentration study used day 1 samples only). Samples were analyzed as-is, without modification; outer skins were not removed for any analysis. The densities of all samples was approximately 1.4 (mass/volume) and the pH values (Orion Research, Beverly, MA) were between 3.20 and 4.30 (Table 01).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>68°B Concord Grape Juice (%)</th>
<th>H₂O (%)</th>
<th>Acid-thinned wheat starch (%)</th>
<th>Soy protein isolate (%)</th>
<th>pH</th>
</tr>
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<td>19.1</td>
<td>11</td>
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<td>3.2</td>
</tr>
<tr>
<td>33% SPI</td>
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<td>4.3</td>
</tr>
</tbody>
</table>

*Shaded rows only used for storage time study

Table 1  Formulations for gummi confections used in this investigation.
Figure 6  Batching kettle, refractometer, and stoving oven used in the manufacture of gummi confections.
4.2 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was used to analyze water distribution within the confections. Three separate batches were tested in quadruplicate for each formulation. Samples, removed centrally from the interior of the confections were weighed to 10 – 15 mg and loaded into platinum TGA pans. A high-resolution thermogravimetric analyzer (Model Q5000, TA Instruments, New Castle, DE) was used for all TGA experiments. Nitrogen was used as the purge gas and set to flow rates of 10 and 25 ml/min for the balance and sample pans, respectively. Samples were heated from ~30 °C to 220 °C using a high resolution (Hi-Res) ramp of 20 °C/min resolved to 3 °C/min whenever the instrument detects a change in weight. Samples were analyzed for both %weight loss and derivative weight loss (%/°C). Derivative weight loss (dTGA) describes the rate of weight loss (%) as a function of temperature (Fessas & Schiraldi, 2001) with peaks indicating temperature ranges of accelerated weight loss. Assuming that all weight loss up to 175 °C is attributed to moisture loss (Fessas & Schiraldi, 2001), the moisture content (MC) was calculated using equation 1.

\[
M.C. = \frac{\text{initial mass (g)} - \text{final mass (g)}}{\text{initial mass (g)}} \times 100
\]

4.3 Texture Profile Analysis (TPA)

Texture profile analyses were conducted using 35 mm compression geometry on an Instron 5542 Universal Testing Machine operating with Bluehill software. For the concentration study, samples were analyzed in quadruplicate for each of three batches for the standard (0% soy), 33%, and 50% soy gummies. The 66% soy protein gummies
could not be evaluated because the samples were destroyed by the texture profiling method. For the storage time study, samples were analyzed in quadruplicate for each of three batches for the standard (0% soy) and 50% SPI samples. Two-step, 50% compression at a rate of 2 mm/sec was performed to simulate mastication (Daubert & Foegeding, 2003). Gumminess, a function of cohesiveness and hardness (Bourne, 2002) was used as one of the traits of comparison between samples and, according to Szczesniak (1962), is the energy required to disintegrate a semisolid food, through mastication, to a point where it can be swallowed.

4.4 Rheological Analysis

All sample analyses were performed using a stress-controlled AR2000ex rheometer (TA Instruments, New Castle, DE). Samples were cut with a circular die to a flat cylindrical shape and then compressed to a thickness of 2 mm with the 20 mm plate geometry. Oscillatory experiments for stress, frequency, and time were performed to elucidate rheological characteristics. Oscillatory stress sweeps (OSS) were performed first at with a range of 0.10 to 4,000 Pa at 1 Hz to determine the linear viscoelastic region (LVR). The critical (yield) stress, the stress at which the material’s deformation was no longer elastic (Liehr, 2000) was obtained. Oscillatory frequency sweeps (OFS) were also performed to determine the linear response range at a stress chosen from within the linear region of the OSS. To determine temperature-dependent behavior, samples were deformed at 1 Hz and constant stress (from the LVR) and heated from room temperature to 100 °C. Lastly, oscillatory time sweeps and creep tests were performed under linear conditions to determine time-dependent behavior. Oscillatory time sweeps (OTS) were used to determine pre-shear requirements for other oscillatory sweeps regarding testing.
within the LVR. Results (data not shown) did not indicate any reproducible pre-shear requirements for other rheometric tests.

4.5 UV-Spec Analysis

The Standard and the 50% SPI treatments were analyzed and compared at day 1 for monomeric anthocyanin content using the haze-corrected pH differential methodology described by Giusti & Wrolstad (2000). Specifically, cyanidin-3-glucoside was used as the basis for comparison between treatments. Analyses were conducted using the HPCORE Chemstation software with the Hewlett-Packard 8453 UV-Vis spectrophotometer.

4.6 Sensory Analysis

An untrained hedonic sensory panel consisting of students and faculty recruited at the Parker Food Science building was conducted to determine the acceptability of fresh gummi confections made with and without soy protein isolate (50% SPI level only). Panelists (n = 40) were asked to score texture and taste on a scale from 1 to 10 (10 = like extremely, 1 = dislike extremely). A sample questionnaire and the participatory letter can be found in Appendices B & C, respectively.

4.7 Statistical Analysis

Data were analyzed using one-way ANOVA with Tukey's multiple comparison method (p = 0.05) in Minitab© 15. Data that failed a test for normality were tested with the Kruskal-Wallis one-way ANOVA test of median equality using SPSS© 18.
5.1 Abstract

The effects of increasing soy protein concentration on the physico-chemical properties of starch confectionery gels were investigated using thermal and rheological analyses. Texture analysis revealed that soy protein decreases hardness and cohesiveness, but with a more dramatic effect on the latter, perhaps demonstrating potential as a texture modifier. Rheological analysis determined that increasing soy protein concentration progressively decreased the elastic properties of the starch network, both by decreasing yield stress and the viscoelastic crossover frequency. High levels of soy protein also created a more homogeneous water population, one which is lost at lower temperatures than standard starch gummi confections. Sensory analysis revealed an improvement in flavor and texture acceptability of gummi confections upon addition of soy protein. Finally, monomeric anthocyanin recovery was greater in gummi confections prepared with soy protein, perhaps by improving heat dissipation during processing.
5.2 Introduction

Gelled confections and candies, collectively referred to as gummies (Edwards, 2000), are a large, stable, and growing business (Young, 1998); As of 2006, industry experts have reported as high as 8.4% growth in the gummi candy sector, mostly attributed to innovative formulations (Anon, 2006). Many of the most common and recognizable gummi confections are typically made with gelatin as the active agent of gelation (Lennox 2002). However, gelatin is not the only option for creating gummi-type candies. Starches may be preferred for being free of animal by-products and the associated concerns (Lennox 2002). However, starch-based confections are sometimes described as “short” an undesirable texture attribute characterized by a firm, brittle gel (Burg, 1998).

Texture is a primary determinant of quality in gelled confectionery products and, due to their relatively simple composition, provide an ideal model for mixed gel investigations (DeMars & Ziegler, 2001). Gumminess and chewiness are texture analysis descriptors that are particularly applicable to gelled confections (Borwankar, 1992). Firmer (short) gummies that are more prone to fracture during mastication are often described as chewy, while softer gummies that dissolve during mastication are described as gummy (DeMars & Ziegler, 2001). Shortness is a texture defect associated with decreased sensory acceptability of gelled confections (Burg, 1998), so quantifying the firmness (shortness) of the gummi confections as it relates to sensory acceptability is prudent. One of the primary uses of soy protein in foods is as a texture modifier (Lusas & Riaz, 1995). Depending on the polysaccharide gel system, soy protein has been shown to have variable results, enhancing (Baeza et al, 2002) or disrupting the gel's structural
integrity (Ryan & Brewer, 2005). Addition of soy protein ingredients to confections was suggested to improve handling properties and stickiness (Rhee, 1994). In a gelled starch-sugar confections, a relatively model gel system, soy proteins would perhaps be beneficial. Therefore, our hypothesis was that soy protein affects the texture of the starch-based confections on a concentration-dependent basis.

To understand the textural changes that were affected upon addition of soy protein, the rheological behavior was assessed. Oscillatory stress sweeps (OSS) reveal the viscoelastic properties and behaviors of foods. Soy protein has been shown to have a pronounced effect on viscoelasticity in gel and food systems (Ryan & Brewer, 2005; Vittadini & Vodovotz, 2003). Oscillating frequency sweeps (OFS) were used to characterize the rheological properties of the confections as a function of time. In these gelled systems, OFS can reveal long-term stability (Rao, 2007), such as tendencies for phase separation, retrogradation, syneresis, or sedimentation. If the addition of soy protein creates a systemic instability, the oscillatory stress and frequency sweeps should reveal differences between the various concentration treatments.

In addition to the solid components of the gummi confection, water is another critical component that affects the overall physical properties. Traditional methods of describing water behavior (water activity, moisture content, and solvent retention capacity) do not adequately describe the distribution and mobility of water in food systems (Slade et al., 1991). Thermogravimetric analysis (TGA) can be used to characterize the changes in physico-chemical properties of a food system as a function of temperature. TGA can be used to track rate of moisture loss and differentiate different water populations within a multi-component food (Fessas and Schiraldi, 2000).
Therefore, TGA will be used to characterize and compare water populations in the gummi confection system and how those are affected by addition of soy protein isolate. The aim of this investigation was to assess the effect of soy protein addition on the physico-chemical properties of a starch confection system. Specifically, the textural, thermal, and rheological properties of starch-based grape juice confections will be evaluated upon incorporation of multiple levels of soy protein. The effect of soy protein on the anthocyanin stability, which is known to be compromised in high-temperature food preparation methods (Patras et al., 2010; Edwards, 2000), will also be assessed in the gummi confections.

5.3 Methodology

Confections were prepared using the ingredients listed in Table 02 as follows: all ingredients were mixed by shaking prior to pouring into the kettle (30 qt., jacketed, plus scraping mixer; Schwepp, Addison, IL 60101). Water and 65 °Brix concord grape juice concentrate (Welch's Foods Inc., Concord, MA 01742) amounts were equivalent across all variables. Acid-thinned wheat starch (Gemstar 1090, Manildra Group USA, Shawnee Mission, KS 66205) and soy protein isolate (Prolisse, Cargill, Inc., Minnetonka, MN 55391) were added to the juice-water solution and then heated to cooking temperature, which was set to and maintained at 100 °C. Confections were subsequently cooked to a final soluble solids content of 70 °Brix (approximately 25 minutes), verified using a high-solids refractometer (Fisher Scientific, Pittsburgh, PA 15275). Confections were then set in Clean Set™ 0736 cornstarch (Cargill Inc., Cedar Rapids, IA 52406) and cured/stoved for 24 hours at 38 °C in an Isotemp® drying oven (200 series, model 215F, Fisher Scientific, Pittsburgh, PA 15275). The final total solids content was
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Table 2. Formulations for gummi confections used in the concentration study.
5.3.1. **Thermogravimetric Analysis (TGA)**

Thermogravimetric analysis (TGA) was used to analyze water distribution within the confections. Three separate batches were tested in quadruplicate for each formulation. Samples, removed centrally from the interior of the confections were weighed to 10 – 15 mg and loaded into platinum TGA pans. A high-resolution thermogravimetric analyzer (Model Q5000, TA Instruments, New Castle, DE) was used for all TGA experiments. Nitrogen was used as the purge gas and set to flow rates of 10 and 25 mL/min for the balance and sample pans, respectively. Samples were heated from ~30 °C to 220 °C using a high resolution (Hi-Res) ramp of 20 °C/min resolved to 3 °C/min whenever the instrument detects a change in weight. Samples were analyzed for both %weight loss and derivative weight loss (%/°C). Derivative weight loss (dTGA) describes the rate of weight loss (%) as a function of temperature (Fessas & Schiraldi, 2001) with peaks indicating temperature ranges of accelerated weight loss. Assuming that all weight loss up to 175 °C is attributed to moisture loss (Fessas & Schiraldi, 2001), the moisture content (MC) was calculated using equation 1.

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5.3.2. **Texture Profile Analysis (TPA)**

Texture profile analyses were conducted using 35 mm compression geometry on an Instron 5542 Universal Testing Machine operating with Bluehill software. Samples were analyzed in quadruplicate for each of three batches for the standard (0% soy), 33%, and 50% soy gummies. The 66% soy protein gummies could not be evaluated because
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5.3.4. **UV-Spec Analysis**

The Standard and the 50% SPI treatments were analyzed and compared for monomeric anthocyanin content using the haze-corrected pH differential methodology described by Giusti & Wrolstad (2000) with a Hewlett-Packard 8453 UV-Vis spectrophotometer plus HPCORE Chemstation software. Specifically, cyanidin-3-glucoside was used as the basis for comparison between treatments.

5.3.5 **Sensory Analysis**

An untrained hedonic sensory panel consisting of students and faculty recruited at the Parker Food Science building was conducted to determine the acceptability of gummi confections made with and without soy protein isolate (50% SPI level only). Panelists (n = 40) were asked to score texture and taste on a scale from 1 to 10 (10 = like extremely, 1 = dislike extremely).

5.3.6 **Statistical Analysis**

Data were analyzed using one-way ANOVA with Tukey's multiple comparison method (p = 0.05) in Minitab© 15. Data that failed a test for normality were tested with the Kruskal-Wallis one-way ANOVA test of median equality using SPSS© 18.

5.4 **Results & Discussion**

5.4.1 **Effects on texture and macro-structure**

The confection hardness (kgF) of the soy treatments analyzed was significantly different (p < 0.05) from the standard (Figure 7). Interestingly, this was not the case with cohesiveness (the attractive forces between like molecules), where the statistically significant decrease was not observed until the 50% soy protein-for-starch substitution
was used (Figure 7, differing letters above error bars designate statistical significance, p < 0.05). The results indicate that while the lower concentration of soy protein has a great impact on hardness, the internal structure is most compromised at higher soy protein concentrations. These results suggest that soy protein can modulate shortness of texture in gelled confections without sacrificing the structural integrity of the system. A similar progressive decrease in hardness was observed by Limroongreungrat and Huang (2007) in pasta upon addition of soy protein. The decrease in hardness could have resulted from direct disruption of the starch network or, as has been shown in bread systems, the hydrated soy protein may have acted as a macro-molecular plasticizer and thereby lowering starch-network stiffness (Vittadini & Vodovotz, 2003). It is important to note that hardness in gelled confections can be misrepresented by case hardening, a surface phenomenon caused by moisture loss that manifests as a “skin” (Ziegler et al., 2003), that is not necessarily representative of the total physical nature of the sample. However, case hardening only became more noticeable as soy protein increased and did not mask the overall softening effect.
Figure 7 Confection hardness and cohesiveness as a function of SPI concentration.
5.4.2 Effects on gel structural properties

Yield stress is the stress that causes permanent deformation (plasticity) of a material. In an oscillatory stress sweep, this is the stress at which a sample is no longer within the linear viscoelastic region (LVR). A typical yield stress is shown in Figure 8. As shown in Figure 9, the first level of soy protein-for-starch substitution (33%) did not exhibit a significantly lower yield stress from the Standard (240 vs. 220 pa, p > 0.05). This may be partly due to the rigid standard's tendency to fracture during the analyses, an analytical issue also observed in the oscillatory frequency sweeps. Elastic modulus decreases significantly upon the addition of soy protein and was low for all soy treatments (Figure 10). Interestingly, the elastic modulus increased significantly (p < 0.05) at the 66% level, but this was thought to be due to a combination of case hardening and sample drying during analysis time based on the progressively decreasing yield stresses as SPI substitution rate increased and the fact that the viscous modulus was greater than the elastic modulus (G”>G'); when this occurs, the material is behaving more like a liquid (Heng et al., 2005). This is in agreement with the results observed with the Instron texture analyzer, where the effects of soy protein substitution were most evident at the higher levels, especially considering the 66% SPI treatment was too liquid-like for comparable analysis with the TPA.
Figure 8  Typical yield stress observed in an oscillatory stress sweep.
This also seems to indicate that soy protein is compromising the rigid starch network rather than forming a gel. Gelation of soy protein isolate is largely dependent on concentration, pH, ionic strength, temperature, and time (Renkema, 2001). This gummy system was low pH (3.2 for the control and 4.1 to 4.3 for the soy protein treatments), low ionic strength (no added salts), relatively low soy protein isolate concentration (3.75% to 7.25%), and prepared at high temperature over a short time period during which water is progressively evaporated. It is suggested that, in this confection system, there is insufficient time and too many interfering solutes for soy protein gelation to occur. Therefore, the soy protein isolate used in this investigation acts as a non-gelling ingredient, meaning there is no interaction between protein units in such a way that a three-dimensional structure is formed (Hermansson, 1985). The extent of denaturation is also not known. Prolisse SPI is made in such a way that there should be minimal protein denaturation; however, DSC analysis of ingredients revealed no endotherms associated with SPI denaturation (data not shown). Gelation of denatured soy protein under gummy manufacturing conditions would require a “bridging” cation, commonly calcium, more time at high temperatures, and/or a higher concentration of soy protein (Wagner et al., 1995) or at least a lower concentration of soluble solutes (Renkam, 2001, Gu et al., 2009). It is also possible that soy protein may not be able to form a continuous gel because the confectionery system's pH is near the isoelectric point (4.5) of this soy protein (Hermannson, 1986; Srejic, 2006; Gennadios et al, 1993). Because soy protein's impact on a carbohydrate gel's physical properties have been shown to be heavily impacted by system pH (Ipsen, 1995), this effect is most pronounced at the 66%
concentration. The pH should not have affected the starch network itself (Russell & Oliver, 1989).

Soy protein may alternatively impede starch hydration during confection preparation and has been observed in other food systems (Ribotta et al., 2005; Molina et al., 1976) due to soy's strong affinity for water (Yao et al., 2006). However, DSC analysis revealed no endothermic peaks that would represent starch crystal melting (data not shown). Alternately or in parallel, the soy protein may simply have interfered with starch gelation. This is supported by the decreasing hardness values observed in Figure 8. Hua et al., (2004) demonstrated that soy protein can indeed inhibit continuous formation of a hydrocolloid network in a carbohydrate system.
Figure 9  Yield stresses (Pa) as a function of soy protein isolate (SPI) substitution.
Figure 10  Elastic modulus (G') as a function of soy protein isolate substitution.
Oscillating frequency sweeps revealed an apparent increase in system dynamics as soy protein concentration increased. The viscous and elastic responses of all treatments displayed varying degrees of frequency dependency (e.g., change in slope as shown in the insert of Figure 11), suggesting that the control and all treatments are subject to time-dependent changes, such as retrogradation. The frequency dependency of the treatments can be differentiated based on the $G'$-$G''$ crossover frequency, which is an indicator of long-term confection system stability. The insert in Figure 11 represents a typical cross-over frequency analysis.

The standard did not always exhibit a crossover point even at high frequencies, as observed previously in more pure starch systems (Byars et al., 2003) although it did exhibit frequency dependency. The observed increase in storage modulus as a function of increasing frequency is to be expected when testing frequency dependency in the linear viscoelastic region (Kohyama & Nishinari, 1993). For comparison purposes, only analyses including a cross-over point were included. Increasing soy protein concentration results in a decrease in the frequency of the $G'$-$G''$ crossover, with the most pronounced decrease occurring between the standard and 33% and also between the 50% and 66% soy protein concentrations suggesting a progressive shift from an elastic to more viscous system. The differences in crossover points observed between the standard and the 33% soy substitution were significantly different ($P < 0.05$). However, $G'$-$G''$ crossover points of the next two substitution levels, 33% and 50%, were not ($P > 0.05$). Lastly, the 66% substitution was significantly lower ($P < 0.05$) than all other treatments. The standard exhibited highly elastic (solid-like) behavior, which was expected due to the high concentration (Rosalina & Bhattacharya, 2001). The progressive decrease in the...
crossover frequency may be due to a destabilizing effect of soy protein, suggesting that, by causing the G’-G” crossover to occur at lower frequencies, soy protein was compromising the starch network (responsible for the elastic properties) of the gel. The greatest changes to the system stability occurred at the 33% and 66%, the latter being the sample which was so destabilized by the high soy protein substitution that it could not be analyzed with the texture analyzer.
Figure 11  Viscoelastic moduli crossover point (G’-G”) as a function of soy protein concentration. Inset graph depicts a typical crossover point.
5.4.3  Effect on water properties and distributions

All confections samples exhibited total weight losses of approximately 50%, of which 25-30% is attributed to sample pyrolysis that occurs as the samples approach 200 °C (Aseeva et al., 2009). This is based on the known final total solids (consistently 78-80%) and the observation that samples removed from the TGA oven at 150 °C were browned, dry, and brittle while samples tested through to 200 °C were ash. This temperature range of 150 to 200 °C corresponds to the second dTGA peak observed in all samples. Weight lost at lower temperatures (less than 100 °C), assumed to be water removed by simple diffusion (Fessas & Schiraldi, 2001) was low, 3 – 6%, with very high sample-to-sample variability. However, it is still possible to differentiate the variables based on the primary water population, in which the rate of weight loss is greatest from approximately 100 to 150 °C. Figure 12 shows that this population of water is relatively uniform except for the 66% SPI treatment, in which the peak terminates at a lower temperature. Zhang (2004) observed that, upon adding soy protein to bread, the derivative weight loss peak shifted to lower temperatures, indicating that the soy system had a weaker water association. Prolisse SPI is considered to be highly soluble (Ohr, 2006; Adams, 2007), but it would seem that its capacity to hold water at high temperatures is low relative to the other components of the gummi system (starch, monosaccharides). Because it still has some ability to hold water, it must not have been rendered fully denatured by the high temperature (100 °C) gummi process (Elmore et al., 2007; Hermannson, 1986). In the confection system, the differences in peak temperatures are only significantly different (p < 0.05) at the highest level, 66%, of soy protein substitution. This indicates that, above 50% SPI, the network in the confection
began to lose its ability to bind water, rather than an incremental decrease as soy protein increased.
Figure 12  Peak termination temperatures of derivative weight loss curves of gummi candies with increasing levels of soy substitutions. Inset graph depicts typical dTGA weight loss peak for 66% SPI variable versus Standard control.
5.4.4 Sensory Analysis

Significant (p < 0.05) improvements to both flavor and texture were observed upon incorporation of soy protein isolate at 50% starch substitution (see Table 3). This supports the texture profile analysis data that shows that soy protein decreases the hardness of the standard, which would be perceived as shortness by a sensory panel. Surprisingly, improved flavor was also reported, which could also be a texture-related effect. Rigid gels been shown to suppress fundamental flavors more than softer gels (Costell et al, 2000; Lethuaut et al, 2003), therefore, it is reasonable to conclude that the soy gummies may have stronger perceived flavor due to lower gel firmness.

<table>
<thead>
<tr>
<th></th>
<th>Code</th>
<th>Texture</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>115</td>
<td>7.56\textsuperscript{a}</td>
<td>7.30\textsuperscript{a}</td>
</tr>
<tr>
<td>50% SPI</td>
<td>698</td>
<td>5.05\textsuperscript{b}</td>
<td>6.23\textsuperscript{b}</td>
</tr>
</tbody>
</table>

*Different letters indicate significant differences (p < 0.05)

Table 3 Mean hedonic scores for Standard and 50% SPI gummi confections.
5.4.5  **UV-Spec Analysis**

As shown in Figure 13, there was a significant increase in MACN survival in the 50% SPI treatment over the Standard. This is unexpected given that anthocyanins can form insoluble complexes with proteins (Edwards, 2000) that would precipitate during sample preparation and also the greater degradation risk associated with higher pH (Laleh et al., 2006). The improved heat dissipation of the 50% SPI, which was evident as a lower cooking viscosity, may have mitigated heat loss of MACN.
Figure 13  Monomeric anthocyanins content, expressed as units of cyanidin-3-glucoside, in the gummi confections versus raw and boiled grape juice concentrate.
5.5 Conclusions

The results of this investigation have demonstrated that addition of soy protein isolate was found to dramatically alter the physico-chemical properties of gummy-type confections. Soy protein was shown to impart an overall softening of gummy candies, most likely due to partitioning the water populations away from, and possibly directly disrupting, the starch gel network. This softening effect, which manifested as decreased hardness, yield stress, and elasticity, became more pronounced as soy protein concentration increased. At higher concentrations, soy protein may completely disrupt the starch network, and may even replace starch as the continuous phase, creating a weak gel held together more by hydrophobic interactions and overall solids concentration. Despite the destabilizing effects that soy protein appeared to impart on the macro-structural properties of gummi confection, it demonstrated potential as a processing aide that improved sensory properties and increased color stability. Sensory analysis determined that addition of soy protein improved hedonic liking scores for texture and flavor.

Increasing soy concentration also caused an apparent increase in the molecular dynamics of the gummy system. The long-term stability of a gummy formulated with soy protein isolate is questionable given the increase in viscous behavior as soy protein concentration increased. Further research to analyze the potential shelf stability of the soy gummies is currently underway.

Soy protein may not be a viable nutritional fortifier of gummy candies given the pronounced effects observed at relatively low concentrations in this investigation. However, the weakening of the gel network due to the inclusion of soy protein could
potentially be used as a processing aide to modulate a specific textural defect, shortness, associated with starch-gelled sugar confections.
CHAPTER 6

EFFECTS OF SOY PROTEIN ON THE STORAGE STABILITY OF STARCH-BASED GELLED CONFECTIONS

6.1 Abstract

The effects of soy protein addition on the storage stability of starch-based gummi confections were investigated using thermal and rheological analytical techniques. Soy protein addition improved the textural stability of the gummy confection by maintaining lower cohesiveness and decreases the confection's resistance to stress. During storage, soy gummi confections demonstrated altered water dynamics, as evidenced by a lower $T_g$ and lower loss temperatures suggesting the potential of using soy protein isolate as a functional ingredient for improved textural profile during storage.

6.2 Introduction

Gelled confections and candies, collectively referred to as gummies (Edwards, 2000), are a large, stable, and growing business (Young, 1998); As of 2006, industry experts have reported as high as 8.4% growth in the gummi candy sector, mostly attributed to innovative formulations (Anon, 2006). Many of the most common and recognizable gummi confections are typically made with gelatin as the active agent of gelation (Lennox 2002). However, gelatin is not the only option for creating gummi-type candies. Starches may be preferred for being free of animal by-products and the associated concerns (Lennox 2002). However, starch-based confections are sometimes described as “short” an undesirable texture attribute characterized by a firm, brittle gel
(Burg, 1998). Furthermore, starch gels have a tendency to retrograde which can manifest as further increase in gel firmness or “shortness” (Leon et al., 1997) which may lower consumer acceptance.

Texture is a primary determinant of quality in gelled confectionery products and, due to their relatively simple composition, provide an ideal model for structure-function investigations (DeMars & Ziegler, 2001). Gumminess and chewiness are texture analysis descriptors that are particularly applicable to gelled confections (Borwankar, 1992). Firmer (short) gummies that are more prone to fracture during mastication are often described as chewy, and softer gummies that dissolve during mastication are described as gummy (DeMars & Ziegler, 2001). One of the many uses of soy protein in foods is as a texture modifier (Lusas & Riaz, 1995). Depending on the polysaccharide gel system, soy protein has been shown to have variable results, enhancing (Baeza et al, 2002) or disrupting the gel's structural integrity (Ryan & Brewer, 2005). Addition of soy protein ingredients to confections was suggested to improve handling properties and stickiness (Rhee, 1994). In gelled starch-sugar confections, a relatively model gel system, soy proteins would perhaps be beneficial to long-term storage stability. In a previous study (Siegwein, 2010), increasing levels of soy protein added to gummy confections were shown to modulate firmness and increase water mobility using rheological and thermal analytical techniques, respectively. Therefore, our hypothesis was that soy protein has a lasting effect on the texture of starch-based confections throughout the defined storage period.

To understand the textural properties that were affected upon addition of soy protein, the viscoelastic behavior obtained through oscillatory stress sweeps (OSS) can be
assessed. Soy protein has been shown to have a pronounced effect on viscoelasticity in gel and food systems (Ryan & Brewer, 2005; Vittadini & Vodovotz, 2003). Analysis of long-term stability of gelled systems utilizing oscillating frequency sweeps (OFS) revealed tendencies for phase separation, retrogradation, syneresis, or sedimentation (Rao, 2007). If the addition of soy protein creates a systemic instability, the oscillatory stress and frequency sweeps should reveal differences in the dynamic rheological properties between the time intervals.

Water is another critical component that affects the overall physical properties of gummy confections. Traditional methods of describing water behavior (water activity, moisture content, and solvent retention capacity) do not adequately describe the distribution and mobility of water in food systems (Slade et al., 1991), particularly in regards to long-term stability (Sablani et al., 2007) and, therefore, quality. Thermal analytical techniques, including differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) can be used to characterize the changes in physico-chemical properties of a food system as a function of temperature. DSC can be used to quantify 2nd order transitions, such as the glassy-rubbery transition, which is associated with an increase in molecular motion of the amorphous phase of materials as they change from the glassy (rigid) to the rubbery (flexible) state (Slade et al., 1991). This state change is driven by plasticizing water (Roos & Karel, 1991), meaning the water itself must be characterized.

The aim of this investigation was to determine how soy protein affects storage stability of gummi confections. Specifically, the textural, thermal, and rheological properties of starch-based grape juice confections will be evaluated at 1, 10, and 20 days.
6.3 Methodology

Confections were prepared using the ingredients listed in Table 02 as follows: all ingredients were mixed by shaking prior to pouring into the kettle (30 qt., jacketed, plus scraping mixer; Schwegge, Addison, IL 60101). Water and 65 °Brix concord grape juice concentrate (Welch’s Foods Inc., Concord, MA 01742) amounts were equivalent across both treatments. Acid-thinned wheat starch (Gemstar 1090, Manildra Group USA, Shawnee Mission, KS 66205) and soy protein isolate (Prolisse, Cargill, Inc., Minnetonka, MN 55391) were added to the juice-water solution and then heated to cooking temperature, which was set to and maintained at 100 °C. Confections were subsequently cooked to a final soluble solids content of 70 °Brix (approximately 25 minutes), verified using a high-solids refractometer (Fisher Scientific, Pittsburgh, PA 15275). Confections were then set in Clean Set™ 0736 cornstarch (Cargill Inc., Cedar Rapids, IA 52406) and cured/stoved for 24 hours at 38 °C in an Isotemp® drying oven (200 series, model 215F, Fisher Scientific, Pittsburgh, PA 15275). The final total solids content was approximately 78-80 °Brix for all formulations, where 80 °Brix is considered standard for gummi-type confections (Koh & Mulvaney, 2004). After curing, candies were cooled to room temperature, gently brushed free of cornstarch, and then heat-sealed in polyethylene bags. Samples were stored at room temperature (~ 20 °C) until analysis at day 1 (fresh), 10, and 20. Samples were analyzed as-is, without modification; outer skins were not removed for any analysis. The densities of all samples was approximately 1.4 (mass/volume) and the pH values (Orion Research, Beverly, MA) were between 3.20 and 4.30 (Table 01).
<table>
<thead>
<tr>
<th>Formulation</th>
<th>68°B Concord Grape Juice (%)</th>
<th>H₂O (%)</th>
<th>Acid-thinned wheat starch (%)</th>
<th>Soy protein isolate (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>69.9</td>
<td>19.1</td>
<td>11</td>
<td>0</td>
<td>3.2</td>
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<tr>
<td>50% SPI</td>
<td>69.9</td>
<td>19.1</td>
<td>5.5</td>
<td>5.5</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Table 4  Formulations for gummi confections used in storage time study.
6.3.1 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to assess the phase transitions in the confections. Three separate batches were analyzed in quadruplicate for both formulations at each interval (1, 10, and 20 days). Samples, extracted centrally from the interior of the gummies, were weighed to 5 – 10 mg and loaded into high-volume stainless steel pans (part #0319-1525, PerkinElmer®, Wellesly, MA) and complementary lids (part #0319-1526, PerkinElmer®, Wellesly, MA) sealed by an O-ring (Lot #2541102, PerkinElmer®, Wellesly, MA). A Q100 DSC equipped with a Refrigerated Cooling System (RCS) (TA Instruments, New Castle, DE) was used for DSC experiments. Nitrogen was used as the purging gas and set to flow rate of 50 mL/min. Indium was used to calibrate the instrument and an empty pan used as a reference. Initially, samples were equilibrated at -50 °C, held isothermally for 3 minutes, and increased 5 °C per minute to 200 °C. All observed thermal events were analyzed using Universal Analysis software (TA Instruments, New Castle, DE).

6.3.2 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) was used to analyze water distribution within the confections. Three separate batches were tested in quadruplicate for all concentrations and at each time interval. Samples, removed centrally from the interior of the confections were weighed to 10 – 15 mg and loaded into platinum TGA pans. A high-resolution thermogravimetric analyzer (Model Q5000, TA Instruments, New Castle, DE) was used for all TGA experiments. Nitrogen was used as the purge gas and set to flow rates of 10 and 25 mL/min for the balance and sample pans, respectively. Samples were heated from ~30 °C to 220 °C using a high resolution (Hi-Res) ramp of 20 °C/min
resolved to 3 °C/min whenever the instrument detected a change in weight. Samples were analyzed for both %weight loss and derivative weight loss (%/°C). Derivative weight loss (dTGA) describes the rate of weight loss (%) as a function of temperature (Fessas & Schiraldi, 2001) with peaks indicating temperature ranges of accelerated weight loss. Assuming that all weight loss up to 175 °C was attributed to moisture loss (Fessas & Schiraldi, 2001), the moisture content (MC) was calculated using equation

\[
M.C. = \frac{\text{initial mass (g)} - \text{final mass (g)}}{\text{initial mass (g)}} \times 100
\]

6.3.3 Texture Profile Analysis (TPA)

Texture profile analyses were conducted using 35 mm compression geometry on an Instron 5542 Universal Testing Machine operating with Bluehill software. Samples were analyzed using texture profile analysis (TPA) in quadruplicate for each of three batches for the standard (0% soy) and 50% SPI samples. Two-step, 50% compression at a rate of 2 mm/sec was performed to simulate mastication (Daubert & Foegeding, 2003). Gumminess, a function of cohesiveness and hardness (Bourne, 2002) was used as the trait of comparison between samples and, according to Szczesniak (1962), is the energy required to disintegrate a semisolid food, through mastication, to a point where it can be swallowed.

6.3.4 Rheological Analysis

All sample analyses were performed using a stress-controlled AR2000ex rheometer (TA Instruments, New Castle, DE). Samples from each treatment and interval were cut with a circular die to a flat cylindrical shape and then compressed to a thickness
of 2 mm with the 20 mm plate geometry. Oscillatory experiments for stress, frequency, and time were performed to elucidate rheological characteristics. Oscillatory stress sweeps (OSS) were performed first at with a range of 0.10 to 4,000 Pa at 1 Hz to determine the linear viscoelastic region (LVR). The critical (yield) stress, the stress at which the material’s deformation was no longer elastic (Liehr, 2000) was obtained. Oscillatory frequency sweeps (OFS) were also performed to determine the linear response range at a stress chosen from within the linear region of the OSS as well as to determine rate-dependent viscoelastic responses. Lastly, oscillatory time sweeps were performed under linear conditions to determine time-dependent behavior. Oscillatory time sweeps (OTS) were used to determine pre-shear requirements for other oscillatory sweeps regarding testing within the LVR. Results (data not shown) did not indicate any reproducible pre-shear requirements for other rheometric tests.

6.3.5. Statistical Analysis

All data were analyzed using one-way ANOVA with Tukey's multiple comparison method (p = 0.05) in Minitab© 15. Data that failed a test for normality were tested with the Kruskal-Wallis one-way ANOVA test of median equality using SPSS© 18.
6.4. Results & Discussion

6.4.1 Texture Profile Analysis

Gumminess results are presented in Figure 14. Gumminess in the standard increased significantly and peaked at day 10, whereas in the 50% SPI the gumminess did not increase significantly until day 20. Both products, therefore, would require more mastication to dissolve after storage. However, since gumminess is a function of both hardness and cohesiveness, the factors contributing to these observations were also analyzed.
Figure 14  Changes in gumminess (N) as a function of storage time.
Hardness results are presented in Figure 15. There was no change in hardness over 20 days for the standard. Hardness increased significantly ($p < 0.05$) in 50% SPI samples at each storage interval studied. For each interval, all samples were significantly ($p < 0.05$) different from each other. During storage, the 50% SPI treatment was observed to have developed a tough skin, possibly due to case hardening caused by surface dehydration of the water associated with soy protein. Soy protein has high water affinity (Vittadini & Vodovotz, 2003), but may be easily removed depending on the food matrix (Smith, 2003). A previous study (Siegwein, 2010) also showed that soy protein could cause the gummi system to more readily lose moisture, although the distribution across the sample was not investigated. Phase separations are common in composite gels (Burey et al., 2009) and may be evidenced by the increased hardness observed in Figure TPA2.
Figure 15  Changes in hardness (kgF) as a function of storage time.
Cohesiveness results are presented in Figure 16. In the Standard gummi confections, cohesiveness increased significantly at each storage interval in the standard. However, there was no change in cohesiveness in the 50% SPI samples over 20 days. These results suggest that while the standard transformed into an increasingly interconnected network over time, most likely due to the relatively high starch concentration (Prokopowich & Biliaderis, 1995), the internal network of the 50% SPI remained relatively static. So while gumminess increased in both treatments, the mechanism by which they increased was different.
Figure 16  Changes in cohesiveness (mm) as a function of storage time.
6.4.2 Effects of soy protein on the dynamic rheological properties over time

Significant differences between standard and 50% SPI samples at all storage intervals were observed with the standard exhibiting a much greater stress resistance, manifesting as a higher yield stress value (Figure 17). The standard samples increased significantly in yield stress at each storage interval, while, in the 50% SPI samples, the yield stress increased significantly only at the 20 day interval. Higher yield stress values are indicative of a system that is progressively becoming more resistant to permanent deformation and perhaps more glassy than rubbery (Ollett et al., 1991), and the relative magnitudes of increasing yield stresses, approximately 1000% vs. 100% for the Standard and the 50% SPI, respectively, reveal that the 50% SPI is much more resistant to the formation of a highly elastic (rigid or “short”) than the Standard.
Figure 17 Change in yield stress (Pa) as a function of time for both the Standard and 50% SPI treatments.
The visco-elastic (G'-G") crossover frequency is the frequency at which the material changes from behaving more like a liquid than a solid (Rao, 2007). Significant differences between the Standard and the 50% SPI sample at each time interval were observed for the cross-over frequency (Figure 18). Within each treatment, significant increase occurred from Day 1 to Day 10 but not from Day 10 to Day 20. Therefore, both treatments exhibited a trend towards more elasticity over time, which was expected in a starch-based gel network (Wang & Sun, 2002). However, the magnitude of crossover frequency increase in the Standard was 71%, and only 38% in the 50% SPI. This indicated that the soy protein both impeded and then delayed rigid network formation, a property observed in soy containing bread (Vodovotz & Vittadini, 2003), which also contained a starch gel component. This, like the stress sweep, indicated that the SPI has a lasting ability to enhance the plasticizing effect of water (Vodovotz and Vittadini, 2003), due to being highly hydrophilic, in the gummi confection matrix (Morales & Kokini, 1999). This may be due to disruption of the starch network, or perhaps by the greater amount of water retained at lower temperatures in the soy-confection, see below, may act as plasticizer of macromolecules.
Figure 18  Change in visco-elastic ($G'$-$G''$) crossover frequency as a function of storage time in both the Standard and 50% SPI treatment.
6.4.3  Effect of soy on water dynamics over time

Derivative weight loss curves, presented in Figure 19a, for the Standard suggests that the water population is progressively more entrapped as storage time increases since it is more difficult to remove. There was a significant (p < 0.05) increase in the peak onset temperature at each time interval. The peak weight loss temperature increased significantly (p < 0.05) from D1 to D20. There was no significant change in total weight loss (moisture content) or low-temperature (<100 °C) weight loss. There is also a significant (p < 0.05) increase in the peak termination temperature at D10 that is not present at D20. This is probably explained by the shoulder on the D10 peak and the small peak that appears at D20 from the ~150 °C to the 175 °C temperature range. This distinct, higher-temperature peak may represent a phase-separated water population, one that appears as a slight shoulder at D1 which becomes more distinct at D10 (Figure 19a). This could be the beginnings of glucose or fructose decomposition, which has been shown to occur in this temperature region (Hurtta et al., 2004), although no melting endotherms were observed with DSC analysis (possibly obscured by the fact that the O-rings regularly failed above 150 °C). It could also be retrograded starch, although the temperature is much higher, 160 to 170 °C, than has been reported in the past for water associated with retrograded starch (Collison & Dickson, 1971), but this gummi confection system is uniquely resistant to water loss due to the high soluble solids content and starch retrogradation rate is concentration dependent (Tolstoguzov, 2003).
Figure 19  Changes in the derivative weight loss curve of the Standard (19a, no soy) and 50% SPI (19b) gummi confections.
A similar trend of increasing temperature of weight loss curves during storage was observed for the 50% SPI treatment (Figure 19b). The peak onset, maximum, and termination points increased significantly (p < 0.05) at D10 and remained stable through D20. There was no change in total weight loss or low-temperature (<100 °C) weight loss. The shift in the peak derivative weight loss at D10 indicates that, like the Standard, the water dynamics within the 50% SPI stabilized by D10. However, the D10 and D20 samples still display a fairly homogeneous water population, one in which all water is lost around 150 °C with peak maxima that are approximately 10 °C lower. There is a high temperature (140 °C) shoulder that begins to resolve at D10, similar to what was observed more dramatically in the Standard. Mir & Nath (1995) showed that, even when controlling for moisture content, soy protein increases the water dynamics of a high-sugar food system at a given temperature.

No melting or crystallization transitions were observed during DSC analysis indicating no detectable “freezable” water remaining in the gummi system, even at higher soy concentrations. There was potentially a glass transition (T_g) that occurs in each variable at about 15 to 35 °C (Figure 20) attributed to the T_g of fructose based on the temperature range and the high concentration of fructose (Shinyashiki et al., 2008; Kalichevsky & Blanshard, 1993). There were no significant differences in the ΔC_p (J/g·°C) of the glass transitions, although the median onset temperature did increase significantly (p < 0.05) in the Standard after 20 days of storage, an increase that was not observed in the 50% SPI. A higher T_g in the D20 Standard may indicate a reduction in molecular mobility, which is supported by the observed increases to cohesiveness during
storage suggesting a shift of the standard towards a less rubbery (more elastic or solid-like) state at room temperature. The lack of any melting endotherms in the ~60 °C range indicates that there is either no detectable crystalline starch (full gelatinization) typical of freshly made starch-based gummi confections (Burey et al., 2009) or that there is not enough plasticizing water to facilitate the reaction (Zeleznak & Hoseney, 1987).
Figure 20  Comparison in the Tg onset temperature between the Standard and 50% SPI gummi confections after 20 days.
6.5 Conclusions

The results of this investigation have demonstrated that addition of soy protein isolate has a long-term ability to alter the physico-chemical properties of gummy-type confections. The starch network disrupting effects observed in the previous study (Siegwein, 2010) continue throughout prolonged storage. During storage, soy gummi confections demonstrated altered water dynamics and decreased firming, although some surface drying (and therefore case hardening) occurred. Overall, the soy gummi confections demonstrated lower overall elasticity or “shortness” suggesting the potential use of soy protein isolate as a functional ingredient for long-term texture stability of these confections.
CHAPTER 7

CONCLUSION

It was hypothesized that adding soy protein to a gummi confection system would have a profound impact on its physico-chemical properties, both as a factor of soy protein concentration and storage time, due to interactions between the main system components (water, sugars, starches, and proteins). To that end, the following aims were addressed.

**Aim 1: To characterize and compare the thermal and rheological properties of starch-based gummies with multiple levels of soy protein incorporation.**

Addition of soy protein isolate was found to dramatically alter the physico-chemical properties of gummi-type confections. Soy protein seems to affect an overall softening of gummy candies, most likely due to partitioning the water populations away from, and possibly directly disrupting, the starch gel network. This softening effect, which manifested as decreased hardness, yield stress, and elasticity, became more pronounced as soy protein concentration increased. At higher concentrations, soy protein may completely disrupt the starch network, and may even replace starch as the continuous phase or render the whole system discontinuous (no ordered associations), creating a weak gel held together more by hydrophobic interactions and overall solids concentration. The softening of the gel network due to the inclusion of soy protein could potentially be used as a processing aide to modulate a specific textural defect, shortness, associated with starch-gelled sugar confections. Increasing soy concentration also caused
an apparent increase in the water dynamics of the gummy system, as evidenced by thermogravimetric analysis results showing decreased moisture loss temperatures at high soy protein concentrations.

**Aim 2: To characterize the changes that occur in gummi candies with and without soy protein during storage.**

During storage, soy gummi confections demonstrated increased water dynamics, manifesting as a resistance to \( T_g \) increase in the DSC thermogram and continued loss of water at lower temperatures during thermogravimetric analysis. Gumminess increased in both treatments, but via different mechanisms; the standard by increasing cohesiveness indicative of a retrograding gel and the 50% SPI by increasing hardness (thought to be an artifact of case hardening). At each storage interval, the soy gummi confections displayed lower overall resistance to stress, lower crossover frequency, and lower relative increases to both over storage. These softening effects were continuances of the effects observed in the first study; the mechanism could be starch network disruption or dilution combined with water partitioning. These results suggest the potential for using soy protein isolate as a functional ingredient for long-term texture stability.

**Aim 3: To determine how soy protein addition affects the sensory acceptability and processing stability of anthocyanins using UV spectroscopy.**

*Sensory Acceptability*

Addition of soy protein was found to significantly improve mean hedonic scores for texture and taste. Both improvements are thought to be caused by a decrease in
shortness, which is in agreement with the texture and rheology results, yielding a smoother texture and enhanced flavor perception.

**UV Spectroscopy**

Despite the disruptive effects that soy protein appears to have on the macro-structural properties of gummi confection, it has demonstrated potential as a processing aide that improved nutritive pigment (color) stability. Monomeric anthocyanin recovery was significantly improved (30% more), in the soy gummi formulation. The mechanism is thought to be based on the effect of viscosity on heat capacity. Addition of soy protein noticeably decreased cooking viscosity, allowing for greater dissipation of heat.

**Future Work**

Characterizing the effects of soy protein addition on gummi confections has yielded a preliminary understanding of how soy protein behaves in a high-solids starch gel and how that changes over time. As a seminal investigation, there is still much research needed to fully understand the molecular nature of the interactions and underlying dynamics that occur upon creation of a mixed/composite gel by addition of soy protein to a high sugar starch network. Specifically, how soy protein’s degree of denaturation and comprising subunits affect physical properties. The possible thermo-protectant action of soy protein on juice anthoycanins during the preparation gummi confections deserves more attention as a potential delivery system. Lastly, it may be worthwhile to map the drivers of sensory acceptability for gummi confections to determine how best to apply texture modifiers like soy protein isolate.
APPENDIX A

[Chemical structure image]
APPENDIX B

THE DEPARTMENT OF FOOD
SCIENCE AND
TECHNOLOGY

College of Food, Agricultural, and Environmental Sciences
Parker Food Science Building, 2015 Fryer Road
Columbus, OH 43210-1007

Phone 614 292-6281
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Participatory Letter

INFORMATION TO SHARE WITH THE PARTICIPANTS.

We would like to invite you to participate in a research study. The purpose of this sensory survey is to determine any differences consumers may notice in starch-based candies and starch+soy candies. You may only participate in this study if you are at least 18 years of age, non-pregnant, and not allergic to soy or wheat. If you wish to participate, please follow the directions on the form given to you and answer the questions. This will take about 5-7 minutes. If you have any questions please ask. Your participation in this study is voluntary; you may decline to participate. If you decide to participate, you may withdraw from the study at any time. If you withdraw from the study before data collection is completed, your data will be returned to you or destroyed.

Your cooperation is greatly appreciated,

Dr. Yael Vodovoz, Associate Professor
DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
THE OHIO STATE UNIVERSITY

Alex Siegwein, Graduate Student
DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY
THE OHIO STATE UNIVERSITY
# APPENDIX C

Taste the samples and check how much you like or dislike each one. Please check the descriptor that best describes your perception of the sample. When you are finished, please slide the tray back through the opening.

<table>
<thead>
<tr>
<th>Appearance, Sample #698</th>
<th>Appearance, Sample #115</th>
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<th>Texture, Sample #115</th>
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</tr>
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