The Functional and Nutritional Benefits of Soy in Snack Foods

Dissertation

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Abstract

Snack foods comprise a larger proportion of the diet in Western cultures than ever before, and many provide very few nutritional benefits. Soy, due to its unique nutritional and functional profile, was hypothesized to increase the nutritional quality of a model snack food, a soft pretzel, as well as increase the frozen shelf life, an important indicator of success in the market. In a narrower focus, the soy pretzel formulation was optimized for intestinal uptake of soy isoflavones while maintaining the physical properties. Using a variety of approaches encompassing physics, material science, food science, in vitro nutrition, and in vivo human nutrition trials, we showed that soy provides nutritional and functional benefits to the soft pretzel, a model snack food.

The dough for bakery products is often frozen for convenient storage and on-site preparation. Retention of the physical properties of the frozen dough is crucial for high consumer acceptability. Addition of soy to dough at 49% slightly mitigated changes in dough and baked soy bread with increased frozen storage time due to the hygroscopic nature of soy protein. The changes in the physical properties of the dough and bread were explained by observing impeded microscale migration of water from the “bound” to “free” state in soy dough compared to wheat dough.

Soy inherently increases the nutritional profile of the soft pretzel by contributing high quality protein, dietary fiber, and soy isoflavones. Glycemic index (GI) was selected as a metric to further characterize the novel pretzel’s nutritional profile because GI has been identified as a broad, key factor that can contribute to promoting or preventing chronic disease [1]. In 12 healthy human participants, the soy soft pretzel exhibited a low GI (39.1 ± 20.4) compared to the wheat pretzel (66.4 ± 15.3), suggesting it to be a more prudent option for people to prevent
hyperglycemia. Notably, the soy pretzel was shown to have equivalent consumer-acceptability ratings as the wheat pretzel.

Soy isoflavones are phytochemicals that provide unique biological effects due to their weak affinity to the estrogen receptor. Therefore, the soy pretzel matrix was further optimized for the uptake of soy isoflavones by enterocytes. A two-fold increase in the proportion of aglycones was achieved by adding raw almonds as the lipid source due to efficient β-glucosidase activity during proofing. Meanwhile, the pretzel maintained moisture content and distribution and exhibited only a slight increase in hardness and chewiness. Approximately 75% of isoflavones from soy-almond pretzels were bioaccessible compared to about 82% in pretzels produced with other types of lipids, but it is hypothesized that this deficit can be overcome by consuming the soy food with lipid [2]. Conversion of the isoflavones from glucosides to aglycones promoted their uptake in an in vitro cell model, but neither the amount nor type of lipid affected isoflavone uptake, metabolism, or transepithelial flux into the basolateral compartment.

In summary, soy ingredients increased the nutritional profile of the soy pretzel by contributing protein, fiber, and phytochemicals and decreasing glycemic index and, additionally, showed to aid in preserving the quality of dough during frozen storage.
Dedicated in loving memory to my grandfather

Francis “Boo” Campbell
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Abbreviations

ANOVA  analysis of variance
ATP  adenosine triphosphate
AUC  area under the curve
BCA  bicinchoninic acid
BMI  body mass index
CCK  cholecystokinin
CP  cross polarized
CRC  Clinical Research Center
$\Delta H$  change in enthalpy
DATEM  diacetyl tartaric esters of mono- and diglycerides
DSC  differential scanning calorimetry
DTG  derivative of the thermogravimetric analysis weight loss curve
$\text{Ext}_{\text{ER}}$  the extension at which the local inflection point is observed in between failure of the two viscoelastic networks in an extensibility experiment
$\text{Ext}_{\text{Rmax}}$  the extension at the maximum resistance in an extensibility experiment
$\text{Ext}_{\text{Rupture}}$  the point at which the material ruptures in an extensibility experiment
$\text{Ext}_{\text{VR}}$  the extension at which the failure of the viscoelastic network occurs in an extensibility experiment
FDA  Food and Drug Administration
FW  “freezable” water
GI  glycemic index
GIP  glucose-dependent insulinotropic polypeptide
GLP-1  glucagon-like protein
HDL  high density lipoprotein
II  insulinemic index
LDL  low density lipoprotein
LPH  lactase phlorizin hydrolase
MAS  magic angle spinning
MR  magnetic resonance
MRI  magnetic resonance imaging
MRP  multi-drug resistance-related protein
NMR  nuclear magnetic resonance
pNP  $p$-nitrophenol
PYY  peptide YY
$R_{\text{max}}$  maximum resistance to extension
$T_{\text{end}}$  the end temperature of the DSC peak
TGA  thermogravimetric analysis
$T_{\text{onset}}$  the onset temperature of the DSC peak
$T_{\text{peak}}$  the peak temperature of the DSC peak
$T_{\text{range}}$  the temperature range of the DSC peak
UFW  “unfreezable” water
USDA  United States Department of Agriculture
VAS  visual analog scale
Chapter 1

Introduction

The developed world is transitioning from a 3-meal-a-day culture to a snack food culture where an average of 20% of calories is consumed from snacks [3,4]. In fact, in January 2012, the Global Industry Analysts declared that the snack food market is expected to reach $334.7 billion by 2015 [5]. The frequency of consumption in combination with the composition of popular snack foods (namely high calorie and low nutrient-density) may be contributing to the obesity epidemic for several reasons: 1) people are consuming more energy than they are expending, and/or 2) the composition of the foods leads to dysregulation of metabolism [3]. Popular snack foods including potato chips, cookies, candy, and even fat-free, carbohydrate-based snacks such as pretzels and crackers often offer very little nutrition apart from calories. Specifically, pretzels are composed of highly processed wheat flour and candy has high amounts of sugar, both of which spike postprandial glycemia and insulinemia levels. Frequent bouts of hyperglycemia have been correlated with insulin resistance and type 2 diabetes which exhibit an increase in prevalence that correlates with snack food consumption [6]. Altering the composition of snack foods with specialty flours and/or other, high nutrient-dense ingredients is a promising solution to maximize both consumer acceptability and health consequences. Soy, specifically, is a particularly promising functional ingredient due to its vast nutritional benefits such as high amounts of high quality protein, fiber, and micronutrients, in addition to its versatility, availability, and low cost [2,7]. It has been shown that soy products are more satiating [8,9] and could help people consume less [10] and also tend to be low in glycemic index [11], thereby
preventing post-prandial hyperglycemia. Hence, the addition of soy to common snack foods could play a role in tackling the world’s growing obesity epidemic [3].

The central hypothesis of this work is that soy can benefit both the nutritional and functional quality of snack foods. In order to address this aim, the soft pretzel presented as an ideal product to use as a model. The soft pretzel dough and the baked product were evaluated on food science and human nutrition levels in order to create a multifaceted illustration of the effects of soy on a wheat-based snack food and to predict their success in the market.

The soft pretzel was chosen from the plethora of snack foods available as the model snack food for these investigations. Soft pretzels are popular snack foods that are consumed in and out of the home, and are increasing in popularity due to consumers’ demand for alternatives to fried snack foods. The crumb of a soft pretzel is more dense and chewy than bread, and easily accommodated soy addition [12]. Soft pretzels were hypothesized to be able to contain high amounts of soy while maintaining consumer acceptability.

A major challenge in incorporating nutritious elements into popular foods is that the new ingredients can drastically change the matrix of the product which, practically, can alter the quality, sensory characteristics, and shelf life of the product. Bread, soft pretzels, rolls, and other bakery products are prepared as frozen dough, shipped to the retail store or event, and then thawed and baked on sight. It is important that functional snack foods exhibit at least the equivalent frozen storage shelf life as the traditional product in order to be considered by many companies as an alternative. However, opportunely, based on the functional properties of soy protein [13] and previous studies with soy bakery products [14,15], soy addition was hypothesized to circumvent challenges in the shelf life of frozen dough. While dough is stored at frozen temperatures, water participates in a thermodynamically favorable migration from starch- and protein-bound states to ice crystals, irreversibly increasing the amount of “freezable” water in the dough, and causing permanent damage to the gluten structure [16,17]. Consequently, the dough requires longer proofing times and produces bread with a smaller loaf volume [17,18].
However, because soy proteins are involved in tight binding interactions with water instead of each other, as gluten proteins are [13], these interactions may reduce the rate of water distribution and be less prone to damage by frozen storage. It is via these mechanisms that soy is believed to reduce the rate of staling of bread [14].

Soy isoflavones are macronutrients that are among the unique components of soy in regard to its health benefits. Epidemiological studies have revealed a strong negative correlation between soy isoflavone intake and hormone-related cancers such as breast and prostate cancers [7,19]. Therefore, it is of interest to optimize the soy pretzel formulation for uptake of the isoflavones in the small intestine. Izumi et al. observed that soy isoflavones are absorbed at a faster rate and to a larger extent when they were in the aglycone form compared to the glucosides form [20]. Preliminary evidence from Zhang et al. showed that addition of almond increased the percentage of isoflavones in soy-almond bread due to natural β-glucosidase activity that is active during proofing [21], offering a relatively easy approach to increase the aglycone content in soy snack foods. Moreover, Walsh et al. found that, due to the hydrophobic nature of isoflavones, especially aglycones, micellarization is necessary for their bioaccessibility and subsequent uptake [2,22]. Micelles are formed spontaneously in the small intestine, facilitated by bile salt surfactants. Composed primarily of lipolysis products of triglycerides, micelles also include amphipathic and hydrophobic compounds from the meal. Due to the hydrophobic effect, additional lipid was hypothesized to facilitate the formation of micelles, thereby potentially accommodating a higher percentage of aglycones in the bioaccessible fraction, and leading to a higher overall uptake of isoflavones.

The overall hypothesis of these investigations was that the addition of soy would improve the nutritional and functional qualities of a soft pretzel. Soy would 1) aid in dough performance during frozen storage by altering the distribution of water and maintaining the integrity of the gluten matrix and 2) increase the soft pretzel’s satiety index and reduce postprandial glycemia due to the higher content of protein and fiber. Thirdly, it was hypothesized that the
bioaccessibility and bioavailability of soy isoflavones can be optimized with further addition of almonds as the lipid source.
Chapter 2

Statement of the Problem

Snack foods that are composed of highly refined carbohydrates are extremely popular despite their lack of healthful nutrients. Herein, a soy soft pretzel was developed and characterized from both food science and human nutrition perspectives.

**Aim 1:** To quantify the changes in the physical properties of soy dough during frozen storage using material science approaches and magnetic resonance techniques.

The hypothesis was that soy would attenuate the physical changes that occur in dough during frozen storage including: i) the reduction in extensibility, ii) the migration of water from the “unfreezable” state to the “freezable” state, and iii) the increase in water mobility. Additionally, it was hypothesized that bread baked from soy dough would exhibit fewer physical differences between fresh and frozen dough compared to the wheat counterpart.

**Aim 2:** To assess the glycemic, insulinenic, and satiety indices of a soy soft pretzel.

Due to the inherent protein and fiber content of soy ingredients, the addition of soy was hypothesized to decrease the glycemic and insulinenic indices and increase the satiety index of a soft pretzel.
Aim 3: To explore the bioaccessibility and bioavailability of soy isoflavones when the delivery matrix contains different types and levels of lipids in an in vitro cell model.

It was hypothesized that utilization of ground whole almonds as a lipid source would shift the isoflavone profile from glucosides to aglycones due to inherent β-glucosidase activity. A higher proportion of aglycones would increase the extent of uptake of isoflavones in an in vitro Caco-2 cell model. Since soy isoflavones are thought to traverse the apical side of the enterocyte membrane from bile-facilitated micelles, increased lipid content was hypothesized to increase bioaccessibility. Furthermore, it was hypothesized that higher levels of fats would increase chylomicron formation within the interior of the enterocyte and facilitate transport of isoflavones across the basolateral membrane.
Chapter 3

Literature Review

In 1919, the first PhD dissertation on soybeans and their nutritional benefits was published by William Bowers at The Ohio State University [23,24]. Bowers examined the macronutrient and micronutrient composition of the soy bean and characterized its components that were nutritious, palatable, and digestible [24]. He found it feasible to mill soybeans into flour and studied the digestibility of “soy cake meal mush” in humans [24]. Prior to his study, bread was being produced with soy flour in a few isolated regions of the world, including France and Austria, for individuals with diabetes. At the time of publication of Bowers’ thesis, the versatility of soy was being discovered in the United States and soy was being processed into bread, crackers, cereals, cheese, milk, confections, and meat substitutes [23]. Of these products, however, soy bread was not received well due to its unconventional dense texture and beany flavor. In the 1940’s and 1950’s, there was a resurgence of interest in soy bread due to its ability to deliver high quality protein to developing countries as a way to combat malnourishment [23]. Unfortunately, in the United States today, soy bread remains relatively rare. The challenge remains to develop a bread with high soy protein content (>3 g soy protein/50 g) that is acceptable to the American population.

The Food and Drug Administration linked soy protein consumption with a decrease in the risk of coronary heart disease in 1999, allowing health claims to that respect on soy foods that contain 6.25 g soy protein per serving, or one quarter of the 25 g/day recommendation [7,25]. A significant body of research has been conducted in the past decade showing that soy may be
beneficial not only in decreasing risk of coronary heart disease, but also in preventing some cancers, relieving menopausal symptoms, and increasing bone strength and density [7,19,26,27 (and references within)]. Despite the recent focused development of various soy-based foods, many commercial products available such as tofu, soy nuts, and soy milk are not amenable to American consumers’ expectations for taste, texture, and general palatability. Substituting soy into familiar products such as bread and snack foods such as pretzels and crackers may prove to be a promising approach to increase soy consumption among Western populations. Optimization of highly acceptable soy bakery products that deliver soy protein, dietary fiber, and isoflavones is one particular approach that shows potential [12,28].

The effects of adding soy ingredients to bread have been characterized in regard to loaf volume and density with the general conclusion that soy leads to a decreased loaf volume and a more dense crumb [14,18,29-35]. The decrease in loaf volume is a result of the high amount of non-gluten protein in the soy flour that entrap water via hydrogen bonding and compromise the gluten’s viscoelastic network [36]. The gluten fraction is overwhelmed by the excess water and non-gluten protein resulting in its inability to properly form disulfide bonds that entrap carbon dioxide, leading to a denser product [37]. While supplementation with gluten improves loaf characteristics, blends with 11% soy flour (wet weight) or more have produced loaves considered unacceptable by North American bread quality standards [38].

In contrast, smaller amounts of defatted soy flour may be useful as a functional ingredient in bread and bakery products. The frozen bakery industry is a 260 billion dollar industry and rapidly growing. Reduction in the quality of the dough during prolonged storage is a large problem that the industry faces. During frozen storage, water migrates irreversibly from the protein and starch components of the dough into ice crystals [17]. Insights on how to ameliorate changes in the matrix during frozen storage may be borrowed from anti-staling research, since both initiatives have a common goal to prevent moisture migration [16] (review). For example, ingredients such as diacetyl tartaric esters of mono- and diglycerides (DATEM) are used to retard
staling and preserve dough during frozen storage [16]. Soy ingredients have been shown to reduce the staling of bread by aiding in the retention of moisture and decreasing the rate of starch crystallization [39]. Hence, it is hypothesized that structural changes in frozen dough can also be impeded with the addition of soy.

This chapter will review the literature on the functional and nutritional effects of soy in bread and bakery products. First, soy will be discussed as a functional ingredient in bread, bakery products, and carbohydrate-based snack foods. Next, the nutritional benefits of soy as a whole ingredient will be discussed. Lastly, the health benefits and methods of evaluation of soy isoflavones specifically will be described.

3.1. Soy as a functional ingredient

3.1.1. Frozen storage of bread dough

Due to high consumer demand for frozen foods, global sales have been steadily increasing and are projected to reach $261.50 billion in 2015 [40]. Sales are increasing despite the fact that, in general, bakery products prepared from frozen dough are of lower quality than those prepared from fresh dough. In bread, loaves have smaller volume and require a prolonged proofing time due to the formation of ice crystals during freezing that damage the dough ultrastructure [17,18]. During frozen storage, water irreversibly migrates from an “unfreezable” state, a state in which the molecules are bound to protein or starch or are simply impeded translationally or rotationally, to the “freezable”, ice crystal-bound state [17,41]. Migration occurs until the unfrozen phase reaches a maximum concentration of solute. The freeze-concentrated phase often vitrifies and the water in this glassy phase comprises the “unfreezable” water (UFW) population. The ratio of “freezable” water (FW) to UFW tends to increase with frozen storage time until the maximum concentration is reached at about 4 weeks [41]. The changes occurring in the water distribution during frozen storage are irreversible, impacting
functionality of the gluten fraction [17]. The effects of globular, hygroscopic proteins, such as soy proteins glycinin and β-conglycinin on the rate of migration during frozen storage remain unknown.

The addition of soy was hypothesized to lessen the deleterious effects of frozen storage of dough. Soy proteins are globular in structure and their amino acid side chains are more hydrophilic than those of wheat gluten. Proteins in soy flour exist in a dehydrated state and quickly bind water when the dough is moistened [13]. Consequently, the dough requires a larger amount of water to achieve optimum workability [28,42]. In contrast to gluten proteins [13], soy proteins are closely associated with water instead of each other. This, these interactions are likely less prone to damage by freezing. Soy protein retains moisture in breads and reduces the rate of bread staling [39] and, therefore, may reduce the rate of moisture migration during frozen storage as well. Moreover, previous studies on frozen parbaked dough have shown that soy addition prolongs fresh-like qualities in microwave applications [43].

3.1.2 Dough analysis methods

3.1.2.1. Thermogravimetric analysis on dough

Thermogravimetric analysis (TGA) is a technique that monitors the precise change in weight of a sample as a function of temperature. This method is often used in food systems, including bread and dough, to determine moisture content and water distribution under the assumption that water is the only major substance that leaves the system as the sample heats from about 25°C to about 200°C [44]. As the temperature increases, water, the molecule with the assumed lowest vapor pressure in the system, vaporizes and the weight of the sample decreases (Figure 3.2). Water exists in dough systems in two major populations- 1) a loosely bound state that vaporizes at temperatures lower than the boiling point, and 2) a state that is hydrogen bound to macromolecules in the system or other water molecules that vaporize near 100°C [44]. By
assessing the derivative weight loss (DTG; Figure 3.2), one can readily distinguish these two populations. Hence, TGA is used to determine the percent of the system that is composed of water as well as provide qualitative information about the state of that water.

3.1.2.2. Differential scanning calorimetry for dough and bread analysis

Differential scanning calorimetry (DSC) is employed in order to characterize physical properties of the components of raw and baked bakery products. DSC directly measures the change in enthalpy of phase transitions. Phase transitions of water, starch, and protein can reveal important information regarding proportions of FW and UFW [45], the amount and types of starch crystallization [46], and the viability of yeast [47], respectively. In frozen dough systems, the shape of the DSC peak has been utilized to observe the process by which freeze-concentrated state was formed [48].

In a DSC experiment, a frozen sample (about -25°C) is heated at a given rate, usually about 10°C/min. The difference in energy requirements required to heat the sample compared to an identical yet empty, air-tight, hermetically sealed pan are monitored. When a phase transition occurs, more heat is required to complete the chemical transition and maintain the increase in sample temperature and a spike is shown on the DSC trace (Figure 3.3). The area under the curve equals the change in enthalpy (ΔH) which can be used to calculate the quantity of ice and, hence, FW [49], amyllopectin crystallization [50], or other crystalline sample components. The temperature that marks the onset of the transition (Tm´) provides useful information. For example, Levine & Slade showed that below this temperature for ice melting, the freeze concentrated state of dough may be formed [46]. Moreover, starch crystallization during staling has been shown to decrease the Tm´, indicating that there are more and/or larger starch crystals [41] or that there are changes in the viscoelastic properties of the dough network [51,52].

DSC has been used extensively to characterize soy bread components in fresh bread and during staling [18,33,39,43,53]. Importantly, DSC was utilized to find that the addition of soy
attenuated the rate of staling in breads by reducing the rate of amylopectin recrystallization and reducing moisture content loss [39]. These properties were hypothesized to manifest during frozen storage, as well, and improve the quality of bread baked from frozen dough.

3.1.2.3. Nuclear magnetic resonance- longitudinal and transverse relaxation for dough analysis

Nuclear magnetic resonance (NMR) is a powerful spectroscopic technique that uses physical properties of nuclei to observe their chemical environments. In dough and many food systems, $^1$H nuclei are probed to assess water mobility [54,55]. Water that is tightly bound to molecules such as starch or gluten has a low water mobility while bulk water has a high water mobility. Water mobility is an important reporter of changes in water distribution upon various processes or changes in dough formulation.

Water mobility can be measured by longitudinal ($T_1$) and transverse ($T_2$) relaxation experiments [54]. A large magnetic field $B_0$ is applied along the z-axis of the sample and each paramagnetic nucleus in the sample aligns with the magnetic field. A perpendicular magnetic field (radiofrequency pulse) is then applied in order to perturb the spins from equilibrium. $T_1$ relaxation times report the time it takes for the nuclear spins to “relax” back to equilibrium, or disperse the magnetization that they absorbed from the radiofrequency pulse. This parameter is related to vibrational, rotational, and translational motions of the protons (that we often assume are in water molecules) [54]. The shortest $T_1$ value occurs when the precession frequency is equal to the Larmor frequency, $\omega_{0z}$, or the frequency at which the proton nuclei precess about the z-axis when in equilibrium in $B_0$. In other words, magnetization transfer among neighboring protons is most efficient at this frequency. As the correlation time of the molecule gets further from the Larmor frequency of the precessing nucleus (either slower or faster) the $T_1$ value increases exponentially (see Figure 3.4). Therefore, samples that are both “solid-like” (low water
mobility) and “liquid like” (high water mobility) have high $T_1$ values. Calculation of the correlation time by using experiments performed at different temperatures or magnetic field strengths or collection of $T_2$ relaxation times can help determine which side of the curve a sample lies.

$T_2$ or spin-spin relaxation experiments report on the loss of coherence of signals in the xy plane. As nuclear spins precess in $B_0$, differences in the environments of local field magnetic fields cause spins to precess at different rates. If the observed nucleus is compartmentalized within the sample and many different yet distinct environments exist, precession coherence will be lost rapidly and spin-spin relaxation times will be short. On the other hand, homogeneous samples or samples that have rapidly fluctuating motions (such as liquids) have a more uniform internal magnetic environment. These samples have high $T_2$ values. Therefore, $T_2$ values increase with increasing liquid-like properties (Figure 3.4).

During frozen storage of dough, water released from the macromolecular matrix shows an increase in water mobility, as measured by NMR [55]. NMR has been used to conduct $T_1$ and $T_2$ relaxation experiments to determine water mobility in both dough and bread matrices [55-59]. Previous studies on parbaked dough have shown that $T_1$ relaxation times decrease proportionally with increasing soy addition in dough implying increased proton mobility [43]. $T_2$ relaxation times are shorter for wheat bread than soy bread, likely due to higher moisture content of the latter [32]. Yi et al. have shown that bread with waxy wheat flour has a higher $T_2$ time than conventional bread and that it retains this higher $T_2$ value during frozen storage [57]. NMR has also shown that yeasted bread that has a higher density (like soy bread) has reduced retrogradation rates [58] and, therefore, may be less prone to damage due to starch crystallization during frozen storage. These findings have led to the hypothesis that globular soy protein binds tightly to water molecules and hinders migration into ice crystals with increased frozen storage, leading to a lower rate of change of both $T_1$ and $T_2$ relaxation times with frozen storage time.
3.1.2.4. Dough extensibility using the Instron texture analyzer

The extensibility of the dough correlates with dough strength and the integrity of the gluten matrix [60,61]. In order to measure extensibility, the sample is clamped on either end and a dough hook, positioned at the midpoint, pulls the dough at a given rate. Total extensibility (in mm) and maximum force (in N) are obtained as well as the shape of the extensibility curve (Figure 3.5) [61,62]. In dough, the extensibility is proportional to the ability for the dough to rise during baking [63]. The extensibility of dough has been used to determine the adequacy of a wheat variety in bakery performance [64] and to describe the development of gluten at various points during dough mixing [61]. The addition of soy has shown to reduce the loaf volume of bread, but the impact had yet to be determined on the extensibility of soy dough.

There is a wealth of information contained in the resultant extensogram (Figure 3.5) [64]. Figure 3.5 shows an extensogram- a graph of force (from the gluten in the dough) vs. extension (or time, since the crosshead moves at a constant velocity). The extensogram is divided into five major regions, divided by local minima or maxima that can be compared among samples and from which information regarding dough strength and gluten integrity can be implied [63,64]. At the commencement of extension, force increases rapidly as the gluten begins to resistance the tug (a). Then, in dough made with weak and intermediate flour, initial failure of the viscoelastic network occurs (ExtVR). This force measurement correlates with strength of the dough. Also in dough made with weaker flour, there are two viscoelastic responses, (a) and (c), divided by a lull in the increase in force (b). There is a local inflection point (ExtER) before the development of the secondary viscoelastic response (c). Dough made with all types of flours exhibit a maximum force value (Rmax) at a particular point of extension (ExtRmax) before gluten failure (d) and rupture (e and ExtRupture). Both Rmax and ExtRmax correlate with loaf volume [63].
3.1.2.5. Solid state nuclear magnetic resonance on dough

$^{13}$C Cross polarization-magic angle spinning (CP-MAS) is a solid state NMR technique that has been used to determine the state of the starch molecules (specifically, the mobility of the carbon molecules) in bread and dough [65]. Packing of starch helices during retrogradation causes a decrease in carbon mobility and an increase in intensity of carbon peaks on a 1-dimensional $^{13}$C spectrum. Baik et al. used $^{13}$C CP-MAS to monitor the crystallization of starch during staling and found that the intensities of $^{13}$C-CPMAS peaks increase with aging due to a more solid-like structure (crystalline packing; Figure 3.6) [65]. Li et al. used CP-MAS to observe the changes in the structure and dynamics of gluten and starch before and after heating and have showed that wheat starch converts from a crystalline structure into an amorphous structure during heating [66]. Before the studies herein, there has been only a spattering of CP-MAS experiments on bread dough, and none to our knowledge that have explored the crystalline structure of starch in dough upon addition of specialty flours.

3.1.3. The role of lipids in soy bakery products

When almonds were added to soy dough at 5.5%, the dough exhibited increased strength and the resultant bread had a higher loaf volume [33]. The changes in physical properties were attributed to the increase in the lipid content, but the contributions of the different components of the almond have not yet been distinguished systematically.

Lipids serve an essential structural role in the production of bakery products. They lubricate the gluten strands and adsorb to the gas-lipid interface of gas cells, allowing the bubbles to develop and be evenly distributed in the crumb [67]. Solid fats (traditionally lard, now more commonly shortening) promote a lighter, more homogenous crumb than liquid oils (review [68]). It is thought that oils do not adhere to the surface of gas cells as well as solid fats do, and therefore promote a less consistent distribution of gas cells and gluten in the dough. An unrefined
lipid source, for example ground nuts, is unique in that it is composed of mostly unsaturated oils, but is present in a solid matrix. It is unknown if oil from ground nut can participate in molecular interactions that promote the growth of gas cells and serve the essential role of lipids in the developing crumb.

Lipids exist as a largely diverse set of molecules, and the chemistry of the various fatty acid side chains may interact differently with the matrix of the dough, leading to differences in gas cell formation, crumb structure, and/or crumb texture. Vegetable shortening, the most common lipid source in bakery products, is partially hydrogenated vegetable oil and is added at about 2-5% [68]. It is solid at room temperature, and is often present in the β or β’ crystal structure [69], meaning that it is more tightly packed in a tilted chain orientation yet can vary between being highly ordered and having some disordered chains, depending on the dispersion of air bubbles and the shortening’s unique fatty acid structure. Shortening has a melting point slightly higher than the proofing temperature (about 40°C), so it is present as a thin semi-solid during proofing. It is hypothesized that the shortening adsorbs to the gluten structure and to the surface of air bubbles, thereby strengthening the dough and retaining gas produced during proofing [68] (and references within). It is thought that the solid structure of the shortening is essential for its function, as the lipid crystals themselves have been observed at the surface between fat and water and fat and air [67]. Canola oil, as a contrasting example, is liquid at room temperature and during proofing, thereby exhibiting no crystal structure. It does not adsorb to the macromolecular matrix or to the interface of gas cells as well as shortening, and thereby does not promote gas retention as well shortening [67,68]. The translational and rotational mobility of the lipid molecules in the canola oil are hypothesized to be greater than in shortening, and therefore hydrophobic interactions may more strongly dictate their orientation. Lipid from almond is mostly unsaturated fat, and would be liquid oil if it were not bound in the lipid matrix. However, it is rotationally and translationally hindered, and it is unknown if it is even available for deposition gluten strands for lubrication or on the surface of gas cells during proofing.
There is some evidence that polar lipids (e.g. phospholipids) improve crumb texture due to interactions with gas cells. In fact, it was observed that the equivalent loaf volume can be achieved with 0.5% polar lipids from oat as with 3.0% shortening [70]. Soybean oil per se contains soy lecithin, a mixture of phospholipids that can improve the extensibility and elasticity of the dough which, in turn, improves loaf volume and crumb texture. It is unknown if the polar lipids within other added lipid sources can aid in crumb development. Polar lipids have not been detected in vegetable shortening [71], and canola oil and almond oil contain a low amount of polar lipids (about 4.5% in canola oil [72] and about 3.7% in mature almonds [73]).

It is important to discern if the lipid component of almonds is responsible for the increased loaf volume of soy bread [33] so that the soy bread formulation can be further optimized for consumer acceptability as well as the health profile.

3.2. Soy as a nutritious ingredient

The nutritional benefits of soy in the animal and human diet have been clearly established. An appreciable amount of evidence exists- epidemiological, laboratory, and human intervention studies- that show that soy provides numerous health benefits for people representing a wide range of health statuses [7,19,25,27].

Soy is unique in that it contains a rich macronutrient profile. Soy is a high quality protein source for the malnourished and for vegetarians, as soy is one of the only non-animal sources of protein that includes all 20 amino acids. Soy is high in essential amino acids such as tryptophan, threonine, and phenylalanine. Of defatted soy flour, 40% is carbohydrates, primarily consisting of sucrose (2.5-8.2%) and the oligosaccharides stachyose (1.4-4.1%) and raffinose (0.1-1.2%) [74-76]. Starch comprises less than 1.0% [77]. Stachyose and raffinose are known to increase the viscosity of the digesta and hinder the rate and extent of digestion [78], but it is debated whether removal of these oligosaccharides improves the digestibility and absorption of soy proteins to a practical extent [76]. A lack of human α-galactosidase causes these oligosaccharides
to be indigestible in the upper small intestine. Instead, they travel to the large intestine where they act as prebiotics, targets for break down by anaerobic microorganisms [77]. These insoluble fibers can promote gut health but may cause abdominal discomfort, including cramps and flatulence, especially when first exposed to the individual [77]. Soy protein has been shown to improve cardiovascular health by decreasing low density lipoprotein (LDL) cholesterol and increasing high density lipoprotein (HDL) cholesterol [7].

There is an increasing number of products available to consumers that are high in soy [7]; any product with 6.25 g soy (one quarter of the 25 g soy/day recommendation by the Food and Drug Administration (FDA) for reducing the risk of cardiovascular disease) is able to include the health claim on their package [25]. Between 1992 and 2010, soy foods sales increased from $300 million to $4.9 billion due to emerging markets and an increase in consumer demand [79]. Most of the soy sales remain in categories such as tofu, soy milk, meat alternatives, energy bars, and soy cheese, yogurt, and ice cream [79]. However, in order for soy foods to benefit a larger subset of the American population, soy needs to be incorporated into a larger variety of foods, including staple foods such as bread and other bakery products.

3.2.1. Soy allergies

It is important when incorporating soy ingredients into common foods to be cognizant of the prevalence of soy allergies. Soy protein is one of the “big 8” food allergies declared by the Food and Agriculture Organization of the United Nations that cause more than 90% of all allergic reactions and must be declared on food labels in the United States [80,81]. At least 16 and as many as 28 soy proteins have been identified as potential allergens with some being more threatening than others [82]. Sensitivity to soy varies among patients and many can tolerate soy lecithin and soy oil; 90% of patients can tolerate up to 400 mg of soy protein [82]. Symptoms tend to be mild and include dermatological (acne, eczema, etc.), gastrointestinal (diarrhea, colitis, nausea, etc.), respiratory (nasal congestion, asthma, etc.), and systemic (low blood pressure, etc.)
reactions [82,83]. A few deaths have been reported from soy allergic reactions; all victims were young (≤18 years old) with known peanut allergies [83]. An attempt is being made to identify and characterize the soy proteins that cause reactions in order to decrease soybean allergenicity, yet the diversity and complexity of soy proteins makes this task difficult [84]. Hypoallergenization of soybean protein may be accomplished by thermal treatment, enzymatic hydrolysis, chemical modification, and/or breeding and genetic modification of the soybean plant [84].

3.2.2. Soft pretzels as a snack food

With the dual aim of increasing the average intake of soy and increasing the nutritional quality of snack foods, a soy soft pretzel was developed for characterization in this thesis project.

It is thought that the pretzel was invented by a 6th century Italian monk who shaped dough to resemble arms crossed in prayer; the three holes represent the Christian Trinity- the Father, the Son, and the Holy Spirit [85]. He gave the pretzels as rewards to children who attended church. The English word “pretzel” is thought to have been derived from the Latin word pretzola, or “little reward” [85]. Historians believe that the Palantine Germans (now known as the Pennsylvania Dutch) brought the soft pretzel to America [85] and, in 1861, Julius Sturgis began the first American pretzel company [86]. Since the pretzels’ introduction, it has been diversified into both soft and hard pretzels as well as serving in snack foods combinations as with cheese or chocolate resulting in $550 million in annual sales [87]. Pretzels became particularly popular with the “fat-free” craze of the 1990’s and remain to be popular in the 2000’s as a “healthy” alternative to fried foods.

With the increased popularity and diversity of pretzels as a snack food and as a form of bread or roll (“pretzel rolls”), a soft pretzel is a promising delivery system for soy. No scientific research had been conducted on soft pretzels to our knowledge at the commencement of this thesis project. However, empirically it was known that pretzels have a denser, moisture crumb
than bread, and soy is successful in delivering a crumb of this texture [39]. Additionally, soft pretzels are often made with white wheat flour and contain few nutrients aside from calories and several B-vitamins [88]. The addition of soy would supplement a host of nutrients that could benefit frequent pretzel consumers.

3.2.3. Glycemic index

One of the properties of typical snack foods that is thought to be detrimental to their nutritional profile, and could be ameliorated with the addition of soy, is that they are composed mostly of refined carbohydrates, thereby having a high GI. The term “glycemic index” was coined in 1981 by David Jenkins, Thomas Wolever, and colleagues as the area underneath the curve of blood glucose vs. time (two hours) after consumption of 50 g of the food of interest compared to 50 g pure glucose [89]. Low glycemic index (GI, <55) [90] meals likely aid in the prevention and control of type 2 diabetes mellitus [91,92] and even cancers such as breast cancer and colorectal cancer due to additional physiological consequences of high blood glucose [93,94]. Although the mechanisms remain controversial, both consumers and health professionals are becoming more aware of GI and are considering it in their food choices and recommendations [90].

The currently available body of evidence has led the World Health Organization, the American Diabetes Association, Diabetes UK, and the Canadian Diabetes Association to endorse GI research in order to elucidate its complex mechanism of potentially preventing and treating diabetes and metabolic syndrome [11]. The interaction between carbohydrates, proteins, fats, and dietary fiber in the diet is important in regard to the speed of digestion and the myriad hormonal processes that are associated with food digestion including glucose uptake. Cellular studies, intervention studies, as well as epidemiological investigations are ongoing to determine the exact role of macronutrients on the body, from maintaining blood glucose levels to insulin resistance to diabetes. Protein and dietary fiber are both believed to decrease the glycemic response to a meal.
and increase satiety by delaying gastric emptying and initiating the secretion of various hormones [95].

Pretzels, that are composed chiefly of white wheat flour, have been shown to have a high glycemic index (83 ± 9) [11]. It was hypothesized that addition of soy, through its contribution of protein and fiber, would decrease the GI of soft pretzels and thereby facilitate control of consumers’ blood glucose.

3.2.3.1. Protein, fiber, and postprandial glycemia

Replacement of carbohydrates with protein leads to greater satiety and a subsequent decrease in caloric consumption [96]. This is the premise behind popular, unconventional diets that advocate high protein and minimal carbohydrates such as the Atkins diet. Supportive evidence shows that protein has an increased thermic effect compared to carbohydrates [96]. Moreover, epidemiological evidence shows an association between higher protein diets, lower blood pressure, and a reduced risk of cardiovascular disease [95].

High protein diets are favorable for people with diabetes and those at risk for diabetes because, in addition to replacing carbohydrates, protein slows gastric emptying, which minimizes postprandial glucose excursions [95]. Chronic, large fluctuations in blood sugar can lead to insulin resistance [91]. Hence, replacement of flour with high protein, specialty flours like soy flour in a soft pretzel (and other high carbohydrate snack foods) has the potential to make soft pretzels an acceptable food item for people with diabetes. Replacement of white wheat flour with specialty flour had not been evaluated to our knowledge.

Protein has shown to be beneficial for attenuating blood glucose after a meal including carbohydrates [97-100]. Protein elicits the release of insulin, which signals corporal cells to take glucose up from the blood stream. When the meal does not contain glucose or glucose polymers, blood glucose is absorbed by body cells and glycemia actually decreases. In liquid form, the addition of 30 g of protein to a 50 g glucose drink decreases the peak rise in blood glucose from
4.5 ± 0.2 mmol/L to 3.1 ± 0.2 mmol/L [100]. In fact, if the meal contains little to no carbohydrates, a proteinaceous meal could decrease blood glucose levels [98,101] since amino acids such as arginine, lysine, and leucine are all pancreatic insulin secretagogues [102]. Although this phenomenon has been shown in healthy individuals, it is debated whether type 2 diabetes mellitus patients can benefit from this attenuation of blood glucose levels by protein [97,99,103,104]. It has been shown that protein stimulates insulin secretion in these patients, sometimes a more intensified response [98]. However, blood glucose may not be lowered in these patients as in healthy adults. Lan-Pidhainy and Wolever put forth that it is not insulin resistance that negates proteins’ glycemia-lowering effect in people with diabetes, and suggest that differences in the secretion of other hormones or in hormone clearance is responsible [103].

It is believed that proteins from different sources elicit varied hormonal responses, as seen by studies with lean beef steak [105] or whey protein [103]. Soy protein, specifically, has been shown to increase insulin secretion in a custard matrix [9]. As part of a low GI diet, 30 g of soy protein per day allowed 26 postmenopausal women (average age 54.6 years old, range 44-65 years) with a body mass index of 27 to 39 kg/m² to significantly reduce their risk factors for cardiovascular disease [106]. It will be important to evaluate the postprandial glycemic response to a soy supplemented soft pretzel in both healthy individuals and people with diabetes independently before recommending them to both populations.

Regardless of the hormonal responses of protein in the system, protein exhibits a higher thermic effect of food than carbohydrates, meaning that the body requires more energy to digest, absorb, and metabolize protein compared to carbohydrates [96]. This is appealing to individuals who are trying to decrease excess body weight. The thermic effect of protein is typically 20-35% of the total amount of energy consumed for protein, compared to 5-15% for carbohydrates [107]. For example, for a 2000 kcal diet, if protein was increased from 15% of total calories to 30%, about 23 extra calories would be burned each day [108]. The increase in energy expenditure for protein processing stems from the fact that protein is not stored in the body as an energy source.
(glucose can be stored directly in the form of glycogen; fat can be stored directly as triglycerides); protein must be metabolized immediately. Eighteen out of the 20 standard amino acids are glucogenic (all but leucine and lysine) and can be converted into glucose. Some are ketogenic and are processed into ketone bodies, depending on the body’s glucose supply. Alternatively, new proteins can be synthesized. All three of these processes require considerable amounts of adenosine triphosphate (ATP) and explain the increased thermogenic effects of protein [96]. Therefore, many dieters, whether they are on an extreme, carbohydrate-restricted diet or a more conventional calorie-reduction diet, are often encouraged to increase their consumption of high protein meals and snacks [96].

Also, but to a lesser extent, dietary fiber was shown to statistically significantly correlate negatively with GI [109]. Wolever found that insoluble dietary fiber had a stronger influence on GI than soluble dietary fiber and found that indigestible carbohydrates such as uronic acid and cellulose could account for 50% of the variability between GIs of 25 foods [109]. When comparing whole apples to a fiber-disrupted apple purée and a fiber-removed fruit juice, it was found that satiety was directly related to the presence and state of the fiber [110].

A number of studies imply that soy specifically has advantageous effects on postprandial glycemia and satiety due to its protein and fiber content. Soy beans (canned, boiled, or in nut form) and foods processed from soy like soy milk, soy yogurt, soy baby formula, Soy Joy® snack bars, and breads made with soy flour all have low GIs (<55) [11]. Soy protein isolate has been shown to increase insulin secretion and satiety [9]. The carbohydrate in soy flour consists of about 18% uronic acid [74] while the carbohydrate of wheat flour is comprised of only about 2.7% uronic acid [111]. Wolever found that uronic fiber and insoluble fiber decrease the glycemic response, suggesting that the fiber in soy ought to decrease the GI of a product in which part of the wheat flour is replaced by soy flour [109]. Collectively, these data suggest that replacing wheat flour with soy ingredients in a soft pretzel will be successful in reducing the glycemic index of the pretzel.
3.2.3.2. Glycemic index methodology

In order to compare the GIs directly among all foods and all studies, Brouns et al. has provided a detailed procedure for GI methodology [112]. The number of subjects for the study should be determined according to the question(s) one is trying to answer. Brouns et al. recommend at least 10 subjects to acquire a GI for a test food with reasonable power and precision [112]. It is understood that an increased number of subjects provides more power and precision and would be able to draw differences between similar test foods, yet will increase the cost of the study. Subjects arrive at the test facility in the morning after an overnight fast and provide a fasting blood sample. Subjects then consume 50 g available carbohydrates (total carbohydrates minus dietary fiber) of either the test food or the standard food. The current recommendation for a standard food is a pure glucose beverage, although white bread has been used for a large proportion of past studies [112,113]. The standard food ought to be tested on at least three separate occasions to assure the precision of the standard area under the curve (AUC) calculation for this food. Capillary finger-stick blood samples are acquired at 15, 30, 45, 60, 90, and 120 min after the start of the meal. Capillary blood is recommended due to its highest sensitivity. The next best type of blood is arterialized venous blood. Normal venous blood is discouraged because variations in ambient temperature and physiological state of the subject can cause fluctuations in glucose uptake into body cells and blood glucose concentrations. An appropriate assay determines the glucose concentration of the blood sample. A graph of glycemia vs. time is generated and the AUC, above the baseline (glycemia at t = 0), is calculated. The glycemic response for the test food is the percentage of the mean of the glycemic responses of the standard food. The GI is calculated by calculating the mean of the glycemic indices for all the subjects.
3.2.3.3. *Postprandial insulin response*

In order to understand the differences between blood composition responses elicited by different foods and within individuals (especially people with diabetes vs. those who do not have diabetes), studies have been conducted measuring GI with the assumption that GI is directly related to insulinemic index (II) [114]. However, this hypothesis has been questioned since the interactions between foods within a meal matrix are multifaceted and extremely complex. Holt *et al.* attempted to develop an equation to calculate II that includes GI, water, sugar, starch, and either fat or protein, with little success [114]. Even within categories of macronutrients, insulin responses can differ; for example, soy protein has shown to reduce postprandial insulin secretion compared to casein in rats [115]. The mechanism behind this phenomenon is currently under investigation, but it appears that glycemic and insulin responses are driven by a combination of mechanical, chemical, and hormonal factors [101,115,116]. In general, protein *per se* is an insulin secretagogue [101] but, since protein causes a decrease in the rate of food digestion, it may lead to a lower postprandial insulinemia maximum. All in all, postprandial insulinemia may vary with food matrix as well as the type and state of the protein and the presence of other macronutrients [100,101,103,114,115].

The determination of the II can be accomplished simultaneously to the GI by an analogous method [112]. The same blood samples can be used for insulin concentration determination; the AUCs of insulinemia vs. time for 2 hours postprandially between the test food and pure glucose are compared.

3.2.4. *Satiety*

The mechanisms by which the state of fullness is achieved are very complex. The state of satiety after a meal involves psychology [117], speed of mechanical breakdown of food [118], hormonal signals to the brain [96], and other mechanisms.
In 2004, Halton & Hu pooled results from 14 satiety studies and reported that 11 out of these 14 studies found that protein increased the subjects feeling of satiety from more than simply its energy content [96]. The mechanism behind this phenomenon is complex and is not currently understood. It appears that protein and amino acids work directly and indirectly to signal to the brain the body’s state of satiety. Both fat and protein directly signal the release of cholecystokinin (CCK), a neuropeptide [119]. CCK then binds to CCK1 (also known as CCK-A) receptors on the vagus nerve and conveys the message of satiety to the brain [116]. Glucagon-like protein 1 (GLP-1) also appears to play a role in signaling satiety but this hormone does not appear to be specific for protein. A third hormone, peptide YY (PYY) is thought to act directly on the hypothalamus to reduce hunger. Protein stimulates the release of PYY to a greater extent than any other macronutrient [120]. It appears that other indirect mechanisms are occurring simultaneously, and can be stimulated differently by different proteins. Pupovac and Anderson observed that intact and hydrolysates of casein and soy both work through the opioid receptor in addition to the CCK1 receptor to increase satiety, but to different extents [8].

It has been shown that increased palatability of a food increases intake, but it is unclear whether palatability affects the resulting state of satiety in either direction [117].

Several studies have assessed the effects of bread formulation or preparation on its satiety with seemingly contradictory results [118,121]. Holt et al. found that a decrease in the energy density of various types of bread (therefore an increase in sample volume) increased satiety [121]. However, Burton & Lightowler prepared bread by varying only the proofing time, thus changing only the loaf volume and crumb density [118]. They observed higher satiety scores with denser breads. This observation was attributed to increased gastric extension by undigested particles, limited starch gelatinization from decreased proofing time, and stronger protein-starch interactions [118]. It has been observed that high protein and high fiber breads had higher satiety ratings [121]. Another satiety study on bread reported that satiety as well as subsequent food intake was not affected by the degree of fat saturation [122].
A soy soft pretzel was expected to have higher protein and fiber content, and a denser crumb and smaller volume compared to the wheat counterpart. It was hypothesized that the hormonal response to the soy soft pretzel would result in a higher level of satiety in the soy soft pretzel compared to the wheat soft pretzel.

3.2.4.1. Satiety index methods

In 1995 the term “satiety index” was coined in attempt to standardize the methodology of satiety studies [10]. The satiety index is analogous to the GI in that the AUC of satiety vs. time (2 hrs) is calculated as the percent of the participant’s same curve for a glucose beverage. However, there are a few key differences as to how the graph is generated. For the y-axis, participants mark on a visual analog scale (VAS) their degree of hunger (Figure 3.7) [123]. Subjects declare their state of hunger/satiety immediately before they consume the test food as the baseline, then they consume an isocaloric portion (1000 kilojoules, or 248 kcal) of the glucose standard or the test food. They declare their degree of hunger/fullness every 15 min after the first bite of food for the first hour and every 30 min for the second hour. For analysis, the study coordinator measures the distance between the participant’s vertical strike and the left end of the line. Because of the subjectivity of the VAS scale, the satiety index is often coupled with a quantitative assessment of subsequent food intake when food is offered ad libitum [10,124].

The VAS has received doubt in its accuracy due to its subjectivity. A second drawback with the VAS is that the results should be analyzed nonparametrically since, although the scale is continuous, equivalent differences between the measured values may not have equivalent meanings since people have a tendency to avoid the extremes [125,126]. However, researchers continue to treat the data parametrically [123]. In general, it is agreed that the VAS is acceptably reliable and valid for satiety experiments, especially when used within-subject under similar circumstances [124]. It is discouraged to compare numerical results between different experiments, however, since the scales, questionnaires, circumstances, and other variables can
lead to systematic differences [124]. For example, participants tend to provide broader satiety responses with paper-based scales compared to electronic appetite rating systems [124]. It is also recommended to use other measures in conjunction with the VAS such as feeding behaviors and changes in blood composition to confirm results [124].

3.3. Soy isoflavones

Soy isoflavones are a renowned component of soy for their anticarcinogenic properties, potential to relieve menopausal symptoms, and potential to increase bone density [7]. Because soy and red clover are the only sources high in isoflavones, and neither of these foods is a large component in the Western diet, soy isoflavone consumption remains very low. Incorporation of soy into common foods such as bread and carbohydrate-based snack foods has the potential to greatly increase the amount of soy isoflavones in the diet of many Americans and other people who consume the Western diet.

3.3.1. Isoflavones in plants

Isoflavones are produced almost exclusively by plants in the Fabaceae (pulse and oilseed) family, most notably the soybean plant, and are considered essential to soybean health [127]. The presence of wounded cells or the presence of compounds produced by various fungi, microbes, or other pathogens, such as wall glucan elicitor from P. sojae, induce isoflavone biosynthesis [128,129]. Isoflavones are biosynthesized in the aglycone form and then stored in the conjugated, β-glucoside form (Figure 3.1). In soy flour, genistein and daidzein and their conjugates compose about 59% and 36% of isoflavones, respectively, while glycitin and its conjugates represent about 5% of total isoflavones [2].
### 3.3.2. Isoflavones and health

Soy isoflavones are now a popular dietary supplement promoted to alleviate a host of physical ailments. It has been shown that isoflavones can attenuate the deleterious physiological changes associated with menopause and prevent the development of cancer [7,19]. The strongest evidence that soy isoflavones are effective at preventing cancer is epidemiological by nature, often comparing East Asian cultures who consume a relatively large amount of soy (average 25-50 mg of isoflavones per day) to Western cultures who consume much less (average <1 mg per day) [130].

Equol, a metabolite of daidzein, has higher biological activity than other isoflavones and may contribute to the health-promoting properties [131]. About 30% of the Western population harbors a microbiota that is capable of metabolizing daidzein into equol, compared to 50-60% of people in China, Japan, and Korea [132]. It has been shown that equol producers celebrate greater improvements in blood lipids compared to non-equol producers [133]. The observation that there is a larger percentage of vegetarians that are equol producers in Western cultures than non-vegetarians suggest that diet affects status, but it wasn’t until 2012 that scientists were able to document a diet-initiated change in equol production status [134,135]. Although it is currently controversial, common belief is that equol status will generally improve the health benefits that soy foods can provide [131].

The structural similarities between isoflavones and estradiol may also play a role in modulating glucose and lipid metabolism, potentially being able to prevent and control the development of obesity and related disorders. Obesity related maladies, collectively called metabolic syndrome, include cardiovascular disease, high cholesterol, type 2 diabetes mellitus, hypertension, and dyslipidemia and may be susceptible to dietary intervention with soy isoflavones [136,137]. Specifically, isoflavones aid in controlling the interpretation of the hypothalamus of hormonal and neural signals to mediate the set-point for energy homeostasis [138]. Isoflavones weakly bind to estrogen receptors and, depending on the tissue, can elicit
estrogenic or antiestrogenic effects [139,140]. The role of estrogens in the prevention of obesity can be appreciated by the observation that postmenopausal women are more prone to the development of visceral obesity than premenopausal women [141-143]. Moreover, estrogen therapy is an effective remedy for certain women for reducing fat mass as well as relieving menopausal symptoms [144]. A host of hypotheses exist regarding how estrogens control glucose homeostasis and modulate insulin sensitivity, but, as a whole, the evidence supports the positive impact of these compounds [145]. Specifically, researchers have shown in rodents that estrogens act directly on the hypothalamic pathway that is stimulated by leptin, the principle hormone that signals satiety [146]. It has also been shown that estrogens can affect adiposicty by directly regulating lipogenesis [138]. Interestingly, it appears that soy isoflavones with soy proteins are more effective in improving blood lipid levels than either component alone [147,148].

Strong epidemiological, in vivo, and in vitro evidence shows that isoflavones have potent biological properties that can prevent the development of cancer by mechanisms including inhibiting cell proliferation, preventing oxidative stress, inducing detoxifying enzymes, and stimulating the immune system [19]. Prevention of cancers related to estrogen such as breast cancer appears to be the most promising for soy intervention due to isoflavones’ estrogenic and antiestrogenic activities [19].

3.3.3. Isoflavones in soy foods

Depending on the variety of soybean, total isoflavone contents can range from 0.1 to 5.0 mg per gram of raw soy bean [149, and references within]. Isoflavones are typically associated with soluble proteins [150]. In soy foods, isoflavones exist in four forms: the aglycone and three glucoside conjugates- the simple β-glucoside, and the acetyl glucoside, and the malonyl glucoside (Figure 3.1) [149]. Processes used to prepare
soy milk, tofu, soy powder, soy flour, and soy nuts tend not to alter the total isoflavone content in foods whereas fermented foods or soy preparations such as soy ice cream and soy sauce contain a lower concentration of soy isoflavones [151]. Processing can also alter the type of isoflavone. High heat and extremes in pH have a tendency to promote hydrolysis of the ester bond that links the malonyl- or acetyl group to the glucose moiety and/or hydrolysis of the acetal bond yielding an aglycone [152].

Because isoflavones require deconjugation for uptake in the small intestine (see §3.3.4), soy bread production has been optimized to convert 67% of simple glucosides to aglycones [153]. It is hypothesized that the processing involved in soft pretzel production, especially the lye dip, will encourage hydrolysis of the isoflavones and yield a product with a higher percentage of aglycones.

### 3.3.4. Isoflavone bioavailability

The bioavailability of the soy isoflavones is essential in order to glean the health benefits. Because of extensive conjugation and intracellular metabolism by enterocytes and then the liver, understanding matrix effects on isoflavone bioavailability is important so that maximal benefits can be gained. The food matrix may hinder (or improve) soy isoflavone absorption and, therefore, effectiveness to lower serum cholesterol concentrations or provide other health benefits [154,155] (see §3.3.2).

#### 3.3.4.1. Bioaccessibility, bioavailability, and metabolism of soy isoflavones

Soy isoflavones, predominately genistein and daidzein, undergo various steps of metabolism between the gut and circulation. In general, the food matrix and the chemical form in which the isoflavone is in the food may not have a large influence on ultimate bioavailability, but
may affect the rate at which the isoflavone is absorbed, the metabolism by gut bacteria, and therefore the net biological effects of the ingested food. Isoflavones are found natively in the soybean in the glycoside form, which is biologically inactive [138] and poorly absorbed compared to the aglycone [20,156,157]. Human β-glycosidases have been identified in the proximal intestine, such as the membrane-bound lactase phlorizin hydrolase (LPH), that cleave isoflavones from the glucose moieties rendering them bioactive [158]. Intestinal bacteria in the duodenum also markedly contribute β-glycosidase activity and it appears that individuals’ populations of bacterial flora may affect net absorption of soy isoflavones [131,132,156,159,160].

The isoflavones that reside in the aqueous fraction, either free or within micelles, are considered “bioaccessible” in that they are able to be transported across the apical membrane of the enterocyte into the cell. Walsh et al. showed that when soy isoflavones are micellarized, at least 90% are bioaccessible and increasing bile concentrations lead to more partitioning of the isoflavones into the aqueous fraction [2]. The food matrix is also predicted to influence soy isoflavone absorption [2,161].

Various studies with soy bread have explored the bioaccessibility and bioavailability of the soy isoflavones [2,153]. It has been shown by Walsh, et al. that micellarization is necessary for optimal bioaccessibility of soy isoflavones from soy bread [2]. Meals higher in fat increase the excretion of bile and formation of micelles. Moreover, meals higher in fat increase the bioaccessibility, and therefore potential total uptake, of carotenoids [162], a more hydrophobic micronutrient, and this may hold true for soy isoflavones as well.

If isoflavones require incorporation into mixed micelles for intestinal uptake [2], the composition of the mixed micelle may affect the rate and/or extent of uptake. For example, smaller micelles have been shown to promote more efficient uptake [163]. Additionally, the composition of the lipid component in the mixed micelle has been shown to affect the uptake of several hydrophobic compounds including carotenoids and cholesterol [162,164,165]. In a study by Yonekura et al., medium chain fatty acids promoted carotenoid uptake from mixed micelles in
a Caco-2 cell model over short- and long chain fatty acids [164]. However, Huo et al. showed that long chain fatty acids maximized incorporation of carotenoids, but did not affect carotenoid uptake into epithelial cells from the micelles [162]. Degree of saturation may also affect incorporation of nutrients into mixed micelles, as Raju et al. showed that carotenoid concentrations in the blood were greater after consumption of carotenoids with oleic acid (18:1) compared to linoleic acid (18:2) [166]. Schneider et al. observed that cholesterol absorption was hindered when stearic acid (18:0) was supplemented to hamsters’ diet and proposed that stearic acid is not incorporated into micelles as well as other lipids [165]. It was unknown what effects the amount of lipid, the length of fatty acid, or the degree of saturation could have on isoflavone bioaccessibility or uptake.

Once inside the enterocyte, the ability of a compound to traverse the basolateral membrane into circulation and be available for tissue uptake and utilization determines its bioavailability. The details of this process are complex and are currently ambiguous [149]. However, isoflavones can be detected in plasma as soon as 30 min after ingestion, suggesting the cleavage, micellarization, and absorption processes are efficient [167]. Once inside the enterocyte, aglycones may be packaged into chylomicrons and excreted through the basolateral membrane into circulation. Alternatively, the aglycones may be re-conjugated into glucuronide or sulfate conjugates which are moved by multi-drug resistance-related protein (MRP) basolateral transporters into the blood stream [149,168]. In isolated rat intestine, high amounts of genistein were converted to the glucuronide conjugate within the enterocyte [161,169]. The observations that 1) very small amounts of free aglycones are found in circulation [170], and 2) extensive conjugation occurs in the liver, support the hypothesis that one or both of these two pathways is being followed [171]. If aglycones are expelled from enterocytes inside chylomicrons, increased lipids in the meal will increase the formation of chylomicrons and, consequently, increase the efficiency of soy isoflavone absorption. Increased bioaccessibility and the bioavailability will benefit consumers with an increased potential for health benefits of isoflavones. It is thought that
the process of isoflavone uptake may behave analogous to carotenoids, where it has been observed that the composition of fatty acids was critical to uptake [162].

Isoflavones undergo extensive first-pass metabolism in the enterocytes and then in the liver, yielding mostly phase II conjugated isoflavones. Once conjugated, they are circulated through the body or excreted in the bile back into the small intestine where, subsequently, they can be reabsorbed [149]. Isoflavones and metabolites can bind plasma proteins and often are stored transiently in tissues that express the estrogen receptor such as the vagina, uterus, and prostate [172]. Due to extensive enterohepatic recycling, exposure of tissues to isoflavones has the potential to be relatively long, before they are excreted by the kidney or in the feces. With that said, about 99% of ingested isoflavones have been to observed to have been excreted after 166 hrs [172].

3.3.4.2. Bioaccessibility methods

The bioaccessibility is the assessment of the amount of nutrient in the aqueous portion of the digesta, available for enterocyte uptake [173]. The development of an in vitro digestion method has allowed for preliminary determination of an estimate of bioaccessibility of various nutrients without invasive human or animal experiments [174,175]. Findings from these studies can then be utilized to design more effective animal and subsequently human studies related to absorption of nutrients. Miller et al. (1981) developed the protocol for studying the bioaccessibility of iron [174] and this protocol has been adopted for studies of carotenoids [175] and further for isoflavones [2].

The in vitro digestion procedure consists of three phases- the oral phase, the gastric phase, and the small intestinal phase. The oral phase for bread and soft pretzels is necessary due to the starchy composition of the matrix, but is not imperative for all in vitro digestion procedures [176,177]. During normal food consumption, the oral phase varies considerably from person to person due to saliva composition [178]. Therefore, the oral phase should be performed either by
one experimenter or be simulated *in vitro*. Then, both procedures converge for the gastric phase which mimics the stomach with a low pH environment and gastric enzymes [2,175]. Lastly, to simulate the small intestinal phase, the pH is increased to 5.3-6.0 with sodium bicarbonate followed by addition of bile extract and pancreatin [2,175]. To assess bioaccessibility, the samples are centrifuged to isolate the aqueous fraction of the chyme [175]. Ions and compounds that are soluble in the aqueous fraction, either in micelles or free in solution, are bioaccessible for enterocyte uptake.

3.3.4.3. **Bioavailability methods**

The bioavailability of a compound is the amount that is transferred across the basolateral side of the enterocyte into circulation, rendering itself available for tissue uptake and utilization [179]. An *in vitro* method for bioavailability has been developed using Caco-2 cells to allow for preliminary results that can then be utilized for animal and then human studies [175].

Caco-2 cells are a heterogeneous human intestinal cell line derived from a colorectal adenocarcinoma [180]. These cells differentiate and polarize spontaneously into a monolayer of enterocyte-like cells [180]. They include apical hydrolytic enzymes, basolateral sodium/potassium-ATPases, and tight junctions between cells [181]. Non-differentiated cells express both colonocyte- and enterocyte-specific proteins, however, as the cells differentiate, colonocyte characteristics are less prominent [182]. At 10-12 days post-confluency, cells morphologically and biochemically resemble enterocytes [181]. The cells require 21-25 days after confluency to develop effective synthesis and secretion of lipoproteins [183]. One key difference between Caco-2 cells and human enterocytes that may be important in lipid bioavailability studies is that Caco-2 cells use the glycerol-3-phosphohate pathway for the synthesis of triacylglycerols while human enterocytes use the monoacylglycerol pathway [184].

It is common to couple *in vitro* digestion experiments with Caco-2 bioavailability experiments to more fully understand the bioavailability of nutrients [181]. Caco-2 cells are
arranged in a compartmentalized apparatus to investigate either cell uptake or cell transport, which includes cell uptake and subsequent efflux across the basolateral membrane (Figure 3.8) [181]. The aqueous phase of the \textit{in vitro} digestion is filtered and added to highly differentiated monolayers of Caco-2 cells [175,181]. After incubation, the apical, intracellular, and basolateral compartments are analyzed for transport and metabolism of the nutrient(s) of interest [181].

The coupled \textit{in vitro} digestion-Caco-2 cell model was utilized herein to investigate the effects of the amount and the source of lipid in a soy soft pretzel on the uptake, metabolism, and efflux of soy isoflavones. It was hypothesized that canola oil would promote micelle formation and therefore bioaccessibility of isoflavones compared to shortening [162]. Additionally, it was hypothesized that, if isoflavones are packaged into chylomicrons within the enterocyte and effluxed into lymph, that a greater amount of lipid in the pretzel formulation would promote more efficient transepithelial flux.

The addition of soy to carbohydrate-based snack foods has the potential to increase the amount of soy consumed by the Western culture. This thesis aims to characterize the functional and nutritional benefits of soy, as well as delineate basic biology associated with soy isoflavone uptake.
Figure 3.1. The three main isoflavones in soy. a) The β-glucoside genistin. The aglycone is called genistein. b) The aglycone daidzein. c) The aglycone glycitein.
Figure 3.2. An example of a TGA curve and its derivative of wheat dough (adopted from [44]).

The red curve shows the total weight of the dough, in mg, during the course of the experiment (linear temperature ramp). The blue curve is the derivative of the weight loss curve, or the rate of weight loss in mg/°C.
Figure 3.3. An example of a DSC curve for soy (●) and wheat (-----) dough for temperatures -50°C to 150°C. The amylopectin peak, which is crucial for analysis of staling, is enlarged in the inset.
Figure 3.4. Schematic of a $^1$H NMR $T_1$ (---) and $T_2$ (●●●●●●) relaxation times as a function of water mobility on a double log scale.
Figure 3.5. Terminology of the dough extensogram obtained by an Instron Universal Testing Machine with a dough hook attachment [64].
Figure 3.6. $^{13}$C CP-MAS NMR spectra of a) fresh and b) stored breadcrumbs. Adopted from Baik et al., 2003 [65].
How full do you feel?

![VAS Scale](image)

**Figure 3.7.** The VAS scale that is completed by participants to report their hunger status at various points throughout a satiety experiment.
Figure 3.8. The arrangement of Caco-2 cell monolayers in the well for the study of nutrient uptake and transport. Figure adopted from Failla and Chitchumroonchokchai, 2005 [181].
Chapter 4

Soy ingredients stabilize bread dough during frozen storage

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**Abstract**

Bread with 48.5% soy ingredients was assessed for quality during frozen storage of the dough. Soy protein was hypothesized to prevent water migration during frozen storage, thereby producing dough that would exhibit fewer structural changes than traditional wheat bread. Wheat and soy bread were baked from dough that was fresh or frozen (-20°C, 2 or 4 wks). Dough and bread were assessed for physical properties including moisture content, percent “freezable” and “unfreezable” water, dough extensibility, and bread texture. The bread was subjected to an untrained sensory panel. The soy bread was denser, chewier, and had a higher moisture content than wheat bread. When baked from fresh or frozen dough, soy bread was rated “moderately acceptable” or higher by 70% of panelists. Soy minimized changes in dough extensibility and resistive force to extension, leading to minimal changes in bread hardness. Although consumers could distinguish between bread baked from soy dough that was fresh or frozen for 4 wks, sensorial and textural data suggested that the rate at which the quality of the soy dough deteriorated was slower than that of wheat dough. In conclusion, the dough of consumer-acceptable soy bread retained quality characteristics during frozen storage slightly better than wheat dough.
4.1. Introduction

Bakery products prepared from frozen dough are typically of lower quality than bread prepared from fresh dough. The loaves have smaller volume and require a prolonged proofing time [17,18]. During frozen storage, water irreversibly migrates from an “unfreezable” state, a state in which the molecules are strongly associated with protein or starch or are simply impeded translationally or rotationally, to the “freezable” state in which they are capable of forming ice crystals [17,41]. Migration occurs until the unfrozen phase reaches a maximum concentration of solute. The freeze-concentrated phase often vitrifies and the water in the amorphous, glassy phase comprises the “unfreezable water” (UFW) population. The ratio of “freezable water” (FW) to UFW tends to increase with frozen storage time until the maximum concentration is reached, about 4 weeks [41]. The changes occurring in the water distribution during frozen storage can irreversibly damage the yeast and the starch and gluten ultrastructure, impacting functionality of the matrix [17,41,52].

Detrimental changes that occur during frozen storage can be circumvented to various degrees by the addition of food additives or specialty flours. For example, dough made with 15% low-amylose, waxy wheat flour has been shown to increase specific volume of loaves produced from frozen dough [57], likely due to increased water absorption of the dough and a reduction in syneresis associated with the amyllopectin fraction. Soy protein has similarly demonstrated increased water holding capacities and interruption of normal packing of dough macromolecules such as gluten protein [13,28] since soy protein can bind covalently (ex. disulfide bonds) and non-covalently (ex. hydrogen bonds) to wheat protein [35]. Because soy protein is involved in tight binding to water instead of other protein molecules, these interactions are more elastic and possibly less prone to damage by freezing. Soy proteins, specifically glycinin and β-conglycinin, are globular in structure and the amino acid composition is more hydrophilic than that of wheat gluten and, since protein molecules in soy flour exist in a dehydrated state [13], they quickly bind water when the dough is moistened. Thus, soy proteins have greater water-binding capacities and
formulations require increased hydration when incorporating them into baked goods [28]. Moreover, a previous study on frozen parbaked dough has shown that soy addition prolongs fresh-like qualities in microwave applications [43].

Among bakers, the addition of soy flour or soy protein isolate to wheat bread at 2-3% has been performed in order to increase the quality of wheat bread from frozen dough in regard to loaf volume and appearance of the crust [185]. Ribotta et al. [52] assessed the effects of the addition of 10% soy flour to wheat dough for frozen storage. However, the goals of their experiment directed them to compare bread made from frozen dough with added soy to fresh wheat dough, and the difference between bread with added soy from fresh vs. frozen dough was not compared. Moreover, differences in the quality of bread made from frozen dough have not yet been evaluated for high soy containing products (about 25% soy ingredients [12]) compared to the wheat counterpart.

Hence, the purpose of this investigation was to characterize the effect of soy addition on the physiochemical properties of frozen dough before and after baking. We hypothesized that, due to the high water-binding properties of soy, water migration would occur at a slower rate in the soy dough during frozen storage compared to traditional wheat dough, resulting in a higher quality product.

4.2. Materials and methods

4.2.1. Dough and bread preparation

A model dough (“wheat dough”) and the soy dough were prepared according to the formulae developed by Zhang et al. [28]. The ingredients used included: wheat flour (Magnifico spring wheat flour, 13.0% protein, ConAgra Mills, Omaha, NE), non-toasted, defatted soy flour (Archer Daniels Mills, Decatur, IL), soy milk powder (Devansoy, Inc., Carroll, IA), sugar, shortening (Crisco® vegetable shortening, J. M. Smucker Co., Orrville, OH), vital wheat gluten (Hodgen Mill, Effingham, IL), Saf-Instant yeast (Lesaffre Yeast Corporation, Sil Fala Lesaffre,
France), sodium chloride, and dough conditioner (Caravan Products Company, Totowa, NJ). The soy dough used soy milk powder and soy flour in an approximate 1:3 ratio so that soy ingredients comprised 48.5% of the dry weight [12]. For the dough experiments, yeast was omitted to avoid production of carbon dioxide that would change the dough matrix during experimental analysis. “Fresh dough” was analyzed the day it was prepared. For “frozen dough”, the samples filled the majority of the volume of 41.25 mL glass jars sealed with parafilm; dough that was used to make bread was placed in gallon-size polyethylene bags. The dough was flash frozen at -40°C for 24 hrs in order to maximize the rate of freezing and then transferred to -20°C for the remainder of the frozen storage period in order to simulate industrial freezing practices [41]. Samples were thawed at ambient temperature the day of analysis. For bread, the dough was placed in a loaf pan and proofed at 39°C for either 30 min (wheat bread) or 60 min (soy bread; CM2000 combination module, InterMetro Industries Corp, Wilkes-Barre, PA). The dough was then baked at 160°C for 60 min (Jet air oven, JA14, Doyon, Linière, Québec, Canada). The bread was allowed to cool for 3 hrs and was placed in a large polyethylene bag overnight. The next morning, the bread was weighed and analyzed for volume using a rapeseed displacement apparatus (method 10-05.01 [186]). It was then sliced into 16 mm slices (Doyon SM302 bread slicer, Linière, Québec, Canada), stored in sealable bags, and analyzed for moisture content, phase transitions between -50°C and 200°C, and texture.

4.2.2. Sensory analysis

Acceptability and difference testing were performed on wheat and soy bread made from fresh and frozen dough. Sensory analyses were approved by the Institutional Review Board at The Ohio State University. All participants were aware of the risks associated with the study and provided written consent. Bread samples were given random, 3-digit numbers and presented in a randomized, counterbalanced fashion at ambient temperature and lighting. Paper-based ballots
were collected and results were analyzed using Compusense® software (Compusense, Inc., Guelph, Ontario, Canada).

In order to test the acceptability of the bread samples, 40 untrained panelists (men and women age 18 to 35) were asked to rate the acceptability of 4 bread samples: 1) soy bread baked from fresh dough, 2) soy bread baked from dough frozen for 2 wks, 3) wheat bread baked from fresh dough, and 4) wheat bread baked from dough frozen for 2 wks. Participants evaluated the samples on a 5-point hedonic scale with the options: “Completely acceptable”, “Moderately acceptable”, “Marginally acceptable”, “Not quite acceptable”, or “Not at all acceptable”.

To establish if consumers could distinguish between bread baked from fresh or frozen dough, 7 triangle tests were performed, 3 in one panel and 4 in a separate panel. For both panels, 40 untrained panelists (men and women age 18 to 35) were recruited. The samples were presented in 3-digit, randomly labeled cups in a random, counterbalanced order. The panelists attempted to identify which 1 of the 3 samples was different from the others. The first panel asked the panelists to distinguish between 1) soy or wheat bread baked from fresh dough, 2) soy bread baked from fresh or 1 month frozen dough, 3) wheat bread baked from fresh or 1 month frozen dough, or 4) soy or wheat bread baked from 1 month frozen dough. The second panel repeated triangle tests 2-4 except the frozen dough was frozen for 2 wks.

4.2.3. Thermogravimetric analysis (TGA)

Total moisture content of the dough and bread samples was measured on the Thermogravimetric Analyzer Q5000 (TA Instruments, New Castle DE). Dough samples of 15-20 mg were spread evenly on the bottom of a TGA pans and analyzed immediately. The chamber was equilibrated at 25°C, held isothermally for 2 min, and subsequently heated to 200°C at 10°C/min. The weight of the sample at 150°C was subtracted from the initial weight to yield the percent of weight lost during heating; this loss was attributed solely to water evaporation [187].

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derivative weight loss (DTG) was calculated by Advantage for the Q Series, version 2.8.0.394 (TA Instruments- Waters LLC, New Castle DE, 2001-2007).

4.2.4. Individual ingredient analysis

Wheat flour, soy flour, soy milk powder, isolated soy protein (PRO-FAM 781, ADM Protein Specialties Division, Decatur IL), and wheat gluten were individually analyzed for rate of weight loss while undergoing a constant temperature ramp. The ingredients were mixed in a 1:1 ratio (by mass) with water and immediately analyzed by TGA as for the dough samples above.

4.2.5. Differential scanning calorimetry (DSC)

Thermal phase transitions were monitored using a Differential Scanning Calorimeter Q100 with a refrigerated cooling system (TA Instruments, New Castle DE). Dough samples of 15-20 mg were pressed to the bottom of a stainless steel DSC pan and hermetically sealed with an O-ring (PerkinElmer, Boston, MA). An empty pan (with O-ring) was used as the reference and indium was used for calibration. The sample was cooled to -50°C, held isothermally for 3 min, and subsequently heated to 150°C at 5°C/min. The percent FW was calculated using the area under the endothermic peak (AUP) near 0°C [18,188]:

\[
\% \text{ FW} = \frac{\text{Enthalpy as determined by AUP near } 0°C (J/g \text{ sample})}{\text{Latent heat of fusion of water } = 333.5 \text{ J/g water}} \tag{4.1}
\]

The percent UFW was calculated by subtracting the percent freezable water from the percent total water [39,49].

4.2.6. Dough extensibility

The Instron Universal Texture Analyzer 5542 (Instron, Norwood MA) was employed to determine uniaxial extensibility of the dough samples. A ball of dough was flattened to 5 mm by a Culver press (Fred S. Culver, Inc.; Summit NJ) in between two plates covered with plastic wrap. The dough rested in the press for at least 2 hrs to allow the gluten to relax [62]. The top
layer of plastic wrap was removed and the dough sat at room temperature for exactly 10 min to eliminate the stickiness and facilitate cutting. Immediately before analysis, a 3.0 cm x 0.5 cm strip of dough was cut and secured by clamps on the Instron. A dough hook attachment then pulled the dough with a crosshead speed of 150 mm/min until the dough ruptured. Bluehill 2 software version 2.17 (Instron, Norwood MA) allowed plotting of force vs. extensibility. The maximum force and extension at maximum load were extracted for analysis. At least 3 replicates were acquired for 3 dough batches, so at least 9 total replicates were acquired per sample.

4.2.7. Texture profile analysis (TPA) of bread

In order to determine hardness, springiness, chewiness, and adhesiveness of bread, TPA was executed on the Instron Universal Texture Analyzer [189]. The cohesiveness was a ratio of the energy to maximum load during the second compression to that of the first compression. The procedure performed a uniaxial, 40% compression with a crosshead speed of 10 mm/min to simulate a double mastication (adopted from the AACC approved method 74-10 [186]). Two adjacent slices from the middle of the loaf were placed one on top of another and four squares were formed with an electric carving knife (Toastmaster, St. Louis, MO) to yield four samples with the dimensions 25 mm x 25 mm x 32 mm. While samples were not being prepared or analyzed they were stored in an air-tight, sealed plastic bag.

4.2.8. Statistical analysis

For moisture content; the proportion of FW or UFW; maximum load; extensibility at maximum load; DSC melt onset ($T_{\text{onset}}$), peak ($T_{\text{peak}}$), end ($T_{\text{end}}$), and range ($T_{\text{range}}$) temperatures; and texture properties, the triplicates for each batch of dough were used in analysis. A mixed model analysis was performed to fit the following model:

$$Y = \text{dough} + \text{batch(dough)} + \text{time} + \text{dough\times time}$$  \hspace{1cm} (4.2)
where $Y = \text{total moisture, FW, UFW, extensibility, maximum force, } T_{\text{onset}}, T_{\text{peak}}, T_{\text{end}}, T_{\text{onset}}$, hardness, springiness, chewiness, adhesiveness, extensibility, or maximum force; dough = wheat or soy; batch(dough) = 1, 2, or 3 for wheat or soy; time = 0, 2, or 4 wks; and dough×time = the interaction between type of dough and length of frozen storage time. The batch(dough) parameter was omitted for the loaf volume statistical analysis. Least-square means for the dough×time interaction were calculated for each analysis using SAS 9.1 (SAS Institute, Cary NC) where $p < 0.05$ was deemed statistically significant. Values are reported as mean ± standard deviation throughout the test.

4.3. Results

4.3.1. Acceptability of bread

Consumer acceptability was assessed for wheat and soy bread prepared from both fresh dough and dough frozen for 2 wks. All samples received acceptability ratings of moderately acceptable or higher from 70% of the panelists (Table 4.1).

4.3.2. Difference testing

In order to determine if consumers could distinguish between soy and wheat bread baked from dough with various lengths of frozen storage time, triangle tests were performed (Table 4.2). Consumers were readily able to distinguish between the soy and wheat bread when the bread was baked from fresh dough or dough frozen for 2 or 4 wks (all $p < 0.001$). When the dough was frozen for 2 wks, consumers could not distinguish between bread baked from the frozen dough or fresh dough for either the soy or wheat bread ($p > 0.05$). However, when the dough was frozen for 4 wks, consumers could distinguish between bread baked from fresh or frozen dough ($p < 0.05$).
4.3.3. Physical properties of dough

Moisture content, percent FW and UFW, and extensibility were assessed on fresh wheat and soy dough and dough frozen for 2 or 4 wks.

As expected from a previous soy bread study [28], soy dough had a higher water content than wheat dough (46.9 ± 0.4% for fresh soy, 42.2 ± 3.5% for fresh wheat; \( p < 0.01 \); Table 4.3). The amount of FW increased in both types of dough with increasing frozen storage time (Figure 4.1a, Table 4.3). After 2 wks frozen storage time, the amount of FW had increased for wheat dough but not soy (\( p < 0.01 \) for wheat, \( p = 0.57 \) for soy). At 4 wks, the FW content for both wheat and soy dough types was significantly greater than fresh dough (\( p < 0.01 \)). Accordingly, the amount of UFW decreased with increasing storage time. While approximately 10% of the water in the wheat dough was UFW at 4 wks frozen storage time, almost none of the water in the soy dough was UFW. The onset of the water melting transition decreased slightly for both the wheat and soy dough with increasing frozen storage time (Table 4.4). The final temperature of the transition peak also slightly decreased and the shape stayed constant (data not shown), therefore the range over which the transition occurred was similar in fresh and frozen dough.

DTG curves indicate the rate of moisture loss for the sample. When observing the DTG curves for soy and wheat dough, the soy dough showed a peak near 55°C and one near 112°C, whereas the wheat samples only displayed the latter peak (Figure 4.2a). Because soy protein was present in soy dough but not in wheat dough, the peak at 55°C was attributed to evaporation of water from soy protein (Figure 4.2b). The temperature at which the peak occurred did not change with frozen storage. The peak near 112°C was at a slightly higher temperature for the wheat dough than the soy dough (\( p = 0.10 \)), and these peaks did not change with frozen storage time (Table 4.3).

A phase transition was present near 75-90°C in the DSC thermogram for fresh and frozen dough. The transition in the soy dough was unimodal and the transition in the wheat dough was bimodal (Figure 4.3). The peak seen in the soy thermogram near 75°C and the peak in the wheat
dough thermogram near 70°C was attributed to melting of the partially crystalline amylopectin structures; the peak in the wheat thermogram at about 90°C was thought to be melting of amylopectin crystalline structures [190,191]. Only a slight deviation from baseline was observed near 85-90°C in the soy dough by DSC thermogram, therefore any fusion of amylopectin crystallites was only barely detectable in the soy dough and unable to be accurately quantified. The $\Delta H$ associated with the amylopectin phase transition was much greater for fresh wheat dough compared to soy dough (0.88 ± 0.12 J/g for soy and 2.53 ± 0.49 J/g for wheat, $p < 0.01$). The size of the peak(s) increased with increasing frozen storage time, implying higher amounts of amylopectin crystallization [191] (Table 4.4). At 4 wks of frozen storage, the amylopectin crystals in the soy dough required 1.12 ± 0.14 J/g and the wheat dough amylopectin crystals required 3.94 ± 0.21 J/g to melt. A phase transition was present in both soy and wheat dough near 110-120°C, which has previously been associated with the melting of amylose crystals [191]. In fresh dough, the amylose crystals required 0.7-0.8 J/g and, after 4 wks frozen storage time, required about 1.1 J/g, implying that the amount of amylose crystallization increased about 50% in 4 wks of frozen storage. The energy required for the amylose crystal melt was not different in wheat and soy varieties.

The extensograms for soy and wheat dough were unimodal (Figure 4.4a) and therefore resembled a hard wheat flour variety [64]. The extension of the fresh wheat dough at the maximum resistance to extension ($\text{Ext}_{\text{Rmax}}$) was approximately 5 times that of fresh soy dough (90.6 ± 12.9 mm for wheat and 16.6 ± 2.8 mm for soy, $p > 0.01$, Figure 4.4b). However, the measured force ($R_{\text{max}}$) that the dough resisted was similar between fresh wheat and soy dough (0.32 ± 0.05 N for wheat and 0.29 ± 0.03 N for soy, $p = 0.57$; Figure 4.4c). At 2 wks frozen storage, the $\text{Ext}_{\text{Rmax}}$ of the wheat dough decreased significantly compared to fresh dough (68.8 ± 6.5 N, $p < 0.01$), and remained unchanged at 4 wks (69.4 ± 15.7 N). The $\text{Ext}_{\text{Rmax}}$ of the soy dough was stable with frozen storage ($p \geq 0.19$). With the decrease in $\text{Ext}_{\text{Rmax}}$, the $R_{\text{max}}$ increased
for frozen wheat dough. The $R_{\text{max}}$ of soy dough did not change between fresh and frozen dough ($p \geq 0.52$).

4.3.4. Physical properties of bread

Moisture content for soy bread ranged between 48-50% as compared to wheat bread with a range 41-43% moisture at all lengths of frozen storage time throughout the experiment ($p < 0.01$ between fresh soy and fresh wheat; $p > 0.39$ between time points of each type; Table 4.5). DTG curves for all bread samples tested depicted a single Gaussian-type curve, as was observed previously for baked goods [18]. DTG curves showed a maximum near 70°C; the soy peak temperature was slightly greater than the wheat peak and increases slightly with frozen storage time (about 6° in 4 wks), while the temperature of the wheat peak was stable with frozen storage time (Figure 4.5).

Figure 1b shows the amount of FW and UFW in bread normalized to the total amount of water in the sample. In soy bread, the water shifted from FW to UFW when the dough was frozen for increasing frozen storage times, yet the FW and UFW content of the wheat bread was stable. The onset of the water melting peak ($T_{\text{onset}}$) was lower for the soy bread and the range of the transition ($T_{\text{range}}$) was greater in soy bread than wheat bread at all of the time points (Table 4.6).

An amylose melt was observed near 110°C-112°C for soy bread and 113°C-116°C in wheat bread on the DSC thermogram (Table 4.6). The enthalpy of the amylose melt was similar between both types of bread and did not show a trend with frozen storage. With the 9 replicates performed on each type of bread at the 3 time points, to yield 18 samples for each type of bread, the soy bread resulted in a much smaller standard deviation for $\Delta H$ than the wheat bread (0.88-1.53 J/g for soy vs. 1.30-1.76 J/g for wheat). Since samples were acquired from random positions in the bread loaves, these results suggest that amylose crystallization may have occurred in different locations in the wheat loaf, perhaps more near the crust and less in the center of the
crumb [192], whereas the soy samples may be more homogenous. Another possibility is that components of the soy formulation may act as plasticizers, preventing formation of amylose crystals [43]. A peak near 70-80°C, which has previously been assigned to amyllopectin crystallization, was too small to quantify, which is typical for fresh breads [39,191].

Fresh soy bread had a hardness of 6.26 ± 1.73 N and was significantly harder than the fresh wheat bread (3.67 ± 1.06, p < 0.01; Table 4.7). The hardness of the wheat bread increased significantly with frozen storage time (p = 0.02 between wheat bread baked from fresh vs. 4 wk frozen dough) but not soy bread (p = 0.15 between soy bread baked from fresh vs. 4 wk frozen dough). The springiness of the bread was similar for the fresh soy and wheat bread (p = 0.33) and was slightly lower in both types of bread made from frozen dough, frozen for either 2 or 4 wks (p < 0.01). Compared to the wheat bread, the soy bread was more cohesive (p < 0.01) and chewy (p < 0.01) than the wheat bread; none of these properties changed with frozen storage time.

The loaf density was significantly greater for the fresh soy bread (0.53 ± 0.03 g/cc) compared to the fresh wheat bread (0.42 ± 0.05 g/cc; p < 0.01; Table 4.7). Soy and wheat bread made from frozen dough was significantly denser (0.62 ± 0.03 g/cc for 2 wk soy, 0.63 ± 0.00 for 4 wk soy, 0.50 ± 0.01 for 2 wk wheat and 0.51 ± 0.01 for 4 wk wheat; p < 0.01 comparing hardness of loaves made from frozen dough to fresh dough), though amount of frozen storage time was not significant (p > 0.72).

4.4. Discussion

With the goal of altering water dynamics and thereby potentially ameliorating the deleterious effects of freezing in dough systems, a consumer-acceptable bread with 48.4% soy ingredients was developed. Untrained panelists were able to distinguish between the wheat and soy breads, though 75% deemed fresh soy bread “completely” or “moderately” acceptable. It is thought that the soy flour-soy milk blend contributed to increased acceptability; in a study by Nilufer et al. [18], soy milk powder was able to add 18% more protein to the formulation while
contributing to a lighter crumb color (as opposed to soy flour, which imparts a darker color at concentrations higher than about 10%) and only slightly changing the physiochemical properties of the matrix.

In order to increase the shelf life and convenience of bread preparation, it is important that the dough undergo minimal changes during frozen storage to maintain high quality. For the fastest preparation time, freezing the fully baked bread would be ideal, but dough has been shown to be much more tolerant to frozen storage than bread [193]. The soy dough formulation presented here appeared to be at least as amenable to frozen storage as the wheat counterpart, with soy addition showing marked physical improvements during frozen storage in the realm of extensibility, resistive force to extension, and hardness of the baked loaf. Differences in bread baked from fresh or frozen dough were not detectable by an untrained consumer panel at 2 wks frozen storage ($p > 0.23$) but were detectable at 4 wks frozen storage time ($p < 0.04$) for both wheat and soy varieties. Data from the triangle tests (Table 4.2) showed that fewer panelists could distinguish between soy bread baked from either fresh or frozen dough compared to wheat bread, suggested that the changes in the soy dough occurred at a slower rate than in the wheat dough.

There was a decrease in loaf volume in bread baked from frozen dough for both soy and wheat varieties (Table 4.7). This suggests that there was damage to the yeast [51], starch, and/or gluten structures [17] and general rearrangement of the dough components. The addition of soy did not attenuate the reduction in loaf volume. Both frozen soy and wheat dough were allowed equivalent time to rise as fresh dough, but it is likely that increased proof time would have resulted in a higher volume loaf for both formulations [17,194]. Accordingly, the bread baked from frozen dough was denser than that baked from fresh dough (about 19% more dense for soy and 21% more dense for wheat). The hardness of the wheat bread increased accordingly, yet the hardness of the soy bread increased only marginally with storage time (8% for soy bread vs. 30%
for wheat bread; Table 4.7). Soy addition to a par-baked flatbread reduced the hardness of the frozen product, suggesting the soy may act as a plasticizer in some carbohydrate matrices [43].

Water evaporation occurred in two distinct phases from the soy dough during TGA (Figure 2) whereas the evaporation of water from the wheat dough displayed a plateau followed by a peak, as has been reported by Daoust et al. [195]. Fessas & Schiraldi performed an extensive analysis of DTG curves on wheat dough samples and attributed water desorption at lower temperatures (roughly ≤ 100°C) to carbohydrate elements and water desorption at higher temperatures (roughly > 100°C) to gluten protein [44]. The raw ingredient analysis in Figure 2b was consistent with these findings. Water in soy containing ingredients was removed at temperatures below 100°C; soy protein isolate, soy milk, and soy flour all display DTG maxima between 50°C and 80°C, suggesting that water that is loosely associated with soy protein. The DTG patterns of wheat dough were bimodal in the analysis by Fessas & Schiraldi [44], possibly because the samples were heated at 2°C/min, allowing sufficient time for starch gelatinization and desorption of water from the gelatinized starch. In this study, small samples sizes (ca. 15-20 mg) were heated at 10°C/min; this rate perhaps prevented substantial water and macromolecular distribution and water loss from the starch at temperatures below 100°C [44].

The quantity of FW increased in both types of dough with frozen storage time as calculated by percent of total sample mass and percent of total water (Figure 1b). This was expected, since water has been observed to migrate from the starch and protein to form ice crystals [17]. The FW fraction of the soy dough was much higher than in the wheat bread, attributed to the increased water added in the formulation and suggesting that even though soy protein binds water strongly [13,28,36], it does not hinder the translational, rotational, and/or diffusible properties of the water. Vittadini & Vodovotz also showed that the added water in a soy bread formulation tended to partition into the FW water fraction [39]. When the dough thawed, the water was possibly phase separated and only during the baking process was there conversion of a portion of FW to UFW. An increase in frozen storage time of the dough
increased the amount of UFW in the baked soy bread (Figure 4.1a). The starch and gluten ultrastructure may have been damaged during frozen storage [17], as well as the yeast [51] and some of the damage may have been irreversible. In bread, air bubbles with uneven size and distribution are surrounded by an uneven matrix of gluten and partially gelatinized starch (some of which is damaged) that can possibly accommodate more water [16] (and references within). Because the gluten matrix was diluted with soy protein in the soy bread, this matrix may be less adaptable to changes caused by frozen storage. In accord with the larger UFW:FW ratio in soy bread baked from frozen dough, a slightly higher temperature was required before water evaporated; the peak water loss according to TGA increased 5.58 ± 4.85°C in soy dough and 1.11 ± 3.28°C in wheat dough (Figure 5).

Differences in moisture content could not be detected between dough and bread due to limitations of the TGA instrument. The dough lost significant amounts of water in the preparation time of the sample, even when this time was minimized. It is estimated from the masses of the pre-baked dough and the final baked bread loaf that approximately 10% of the loaf mass was lost during baking. This loss in weight is possibly from water evaporation and, to a smaller amount, carbon dioxide evolution from yeast.

Amylopectin crystallization was evident in both soy and wheat dough upon frozen storage, as observed by increasing $\Delta H$ values for the amylopectin melt with frozen storage time (Figure 3). The wheat dough showed two overlapping yet distinct peaks, as seen by Schiraldi et al. [50] and Ribotta et al. [52]. These peaks were attributed to melting of partially crystallized amylopectin and melting of tightly packed amylopectin polymers, respectively. The peak at lower temperature was present in both types of dough, however it appeared that the amylopectin concentration in the soy dough was insufficient to allow formation of a tightly packed crystal. Alternatively, the amount of water in the system could have affected the process of amylopectin packing [196]. Re-crystallization of starch polymers can contribute to deterioration of the quality of frozen dough, and it appears that changes in soy dough, as seen by $\Delta H$ of the amylopectin and
amylose melts, occurred at a smaller rate and extent than in the wheat dough (Table 4.4). Similarly, soy has been shown to attenuate the rate of staling due in part to its ability to reduce the extent of amylpectin recrystallization [39].

The extensibility of the dough directly correlates with loaf volume [63] since it is a measure of how far the dough can extend (either under pressure of the gas bubbles or by the texture analyzer) before rupture. With the decrease in $\text{Ext}_{R_{\text{max}}}$, the $R_{\text{max}}$ increased for wheat dough with frozen storage time. Anderssen et al. observed the same phenomenon when comparing different varieties of wheat flour [64]. The soy dough, however, does not appear to fit into this model; it has a particularly large $R_{\text{max}}$ for its relatively small $\text{Ext}_{R_{\text{max}}}$. The soy protein replaced some of the gluten protein in the formulation and also diluted the amount of gluten protein cross-linking. The soy protein may have been forming strong yet inelastic bonds with the wheat protein [197]. While frozen storage deteriorates the gluten network [51], the soy-wheat bonds appeared to be unaffected by frozen storage.

The dough samples did not include yeast, however, in the bread baked from dough the soy proteins may have provided protection to the yeast cells in the sub-zero temperatures. Izawa et al. found that *S. cerevisiae* cultured with soy peptides had a higher tolerance to freeze-thaw cycles than the control medium and contributed to bread with higher loaf volume [198].

4.5. Conclusions

Hydrocolloids and emulsifiers were not used in this formulation in order to focus on the changes imparted by soy addition alone, however they may improve the frozen shelf life of the soy dough in addition to increasing extensibility and loaf volume. Attenuation of the decrease in loaf volume with soy protein addition has been shown with phosphatidylcholine, sodium stearoyl-2 lactate, and others [199] (and references within). Emulsifiers and hydrocolloids also are popular in maintaining quality characteristics of frozen wheat dough [193]. The effects of additives on soy dough remain to be explored.
In conclusion, soy addition may have reduced the rate at which frozen storage depreciates dough quality. The soy protein, while adding possible nutritional benefits, may functionally act as a plasticizing agent by loosely binding water. While not preventing water migration during frozen storage, the extra water reduced the crystallization of amylopectin and attenuated the increase in hardness as seen in bread baked from frozen rather than fresh dough.

4.6. Acknowledgments

We would like to thank Zoë Edmiston and Audrey Owens for help with sample preparation and data collection.
Figure 4.1. The percent of the total moisture content that is FW and UFW in dough (a) and bread (b). Soy FW is black, soy UFW is dark gray; wheat FW is light gray, wheat UFW is white.
Figure 4.2. An example DTG curve of a) fresh soy (black) and wheat (gray) dough and b) the raw ingredients in a 1:1 ingredient:water mixture.
Figure 4.3. Typical DSC thermograms are used to identify amylopectin and amylose phase transitions. The top two curves are from fresh dough, the bottom two curves are from dough frozen for 2 wks; black lines are from soy dough and gray lines are from wheat dough.
Figure 4.4. a) Example extensograms for fresh soy (black) and fresh wheat (gray) dough, b) the effects of frozen storage time on the extension at the maximum measured force (Ext$_{R_{max}}$) for wheat (○) and soy (●) dough, c) the maximum resistive force (R$_{max}$) the dough during extension for wheat (○) and soy (●) dough.
Figure 4.5. Example curves showing the rate of weight loss (DTG) for soy (black) and wheat (gray) bread baked from fresh dough (solid lines) or dough that was frozen for 4 wks (dashed lines).
<table>
<thead>
<tr>
<th>Sample</th>
<th>Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bread from fresh dough</td>
<td>95%</td>
</tr>
<tr>
<td>Wheat bread from frozen dough</td>
<td>82%</td>
</tr>
<tr>
<td>Soy bread from fresh dough</td>
<td>75%</td>
</tr>
<tr>
<td>Soy bread from frozen dough</td>
<td>70%</td>
</tr>
</tbody>
</table>

**Table 4.1.** The percent of panelists that rated the bread “completely acceptable” or “moderately acceptable”. The “frozen dough” was frozen for 2 wks before baking.
Comparison of dough types | 0 wks (fresh) | 2 wks frozen | 4 wks frozen
--- | --- | --- | ---
Fresh wheat vs. fresh soy | $p < 0.001$ |  |  
Frozen wheat vs. frozen soy |  | $p < 0.001$ | $p < 0.001$  
Fresh wheat vs. frozen wheat |  | $p = 0.23$ | $p = 0.01$  
Fresh soy vs. frozen soy |  | $p = 0.47$ | $p = 0.04$  

**Table 4.2.** Triangle tests were performed to observe if consumers could distinguish between soy and wheat bread and soy or wheat bread baked from dough with various levels of frozen storage time ($n = 40$).
<table>
<thead>
<tr>
<th>Dough Type</th>
<th>Frozen Storage Time</th>
<th>Total Water Content (%)</th>
<th>Peak Water Loss 1 (°C)</th>
<th>Peak Water Loss 2 (°C)</th>
<th>FW (%)</th>
<th>UFW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>0 wks</td>
<td>46.9 ± 0.4 a</td>
<td>58.9 ± 6.6 a</td>
<td>111.1 ± 1.7 a</td>
<td>37.3 ± 6.2 a</td>
<td>9.6 ± 6.2 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>46.7 ± 0.7 a</td>
<td>54.6 ± 5.5 a</td>
<td>111.3 ± 2.2 ab</td>
<td>36.8 ± 5.7 a</td>
<td>9.9 ± 5.7 a</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>47.4 ± 0.8 a</td>
<td>55.1 ± 3.2 a</td>
<td>113.1 ± 2.3 b</td>
<td>46.7 ± 2.9 b</td>
<td>0.2 ± 2.9 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>0 wks</td>
<td>42.2 ± 3.5 b</td>
<td>-</td>
<td>112.8 ± 1.9 ab</td>
<td>23.7 ± 3.8 c</td>
<td>18.5 ± 3.8 c</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>40.8 ± 0.2 c</td>
<td>-</td>
<td>112.2 ± 2.3 ab</td>
<td>32.8 ± 2.1 d</td>
<td>8.0 ± 2.1 a</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>40.9 ± 0.3 bc</td>
<td>-</td>
<td>113.1 ± 2.8 b</td>
<td>34.5 ± 1.5 bd</td>
<td>6.4 ± 1.5 a</td>
</tr>
</tbody>
</table>

Table 4.3. The total water content of soy and wheat dough, the peak(s) of the DTG, and the FW and UFW percentages of the total dough mass.
<table>
<thead>
<tr>
<th>Dough Type</th>
<th>Frozen Storage Time</th>
<th>$\Delta H$ of Water Melt (J/g)</th>
<th>$T_{onset}$ (°C)</th>
<th>$T_{peak}$ (°C)</th>
<th>$T_{range}$ (°)</th>
<th>Temp. of Amylopectin Melt (°C)</th>
<th>$\Delta H$ of Amylopectin Melt (J/g)</th>
<th>Temp. of Amylose Melt (°C)</th>
<th>$\Delta H$ of Amylose Melt (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>0 wks</td>
<td>124.54 ± 20.74 a</td>
<td>-27.62 ± 1.32 a</td>
<td>-1.05 ± 1.45 a</td>
<td>42.05 ± 3.20 a</td>
<td>77.18 ± 1.41 a</td>
<td>0.88 ± 0.12 a</td>
<td>112.90 ± 1.48 a</td>
<td>0.82 ± 0.16 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>122.77 ± 19.16 a</td>
<td>-27.52 ± 1.59 a</td>
<td>-1.32 ± 1.29 a</td>
<td>41.97 ± 2.53 a</td>
<td>77.35 ± 1.84 a</td>
<td>0.92 ± 0.14 ab</td>
<td>113.56 ± 1.53 ab</td>
<td>0.89 ± 0.20 a</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>155.68 ± 9.59 b</td>
<td>-29.03 ± 0.55 b</td>
<td>-2.88 ± 0.56 b</td>
<td>42.17 ± 2.97 a</td>
<td>75.49 ± 0.72 b</td>
<td>1.12 ± 0.14 b</td>
<td>111.11 ± 0.68 b</td>
<td>1.11 ± 0.09 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>0 wks</td>
<td>79.16 ± 12.55 c</td>
<td>-24.16 ± 2.30 c</td>
<td>0.05 ± 0.86 c</td>
<td>38.63 ± 1.99 b</td>
<td>76.16 ± 1.10 b</td>
<td>2.53 ± 0.49 c</td>
<td>117.59 ± 0.90 c</td>
<td>0.72 ± 0.21 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>109.46 ± 6.99 d</td>
<td>-27.45 ± 0.98 a</td>
<td>-2.63 ± 0.42 b</td>
<td>38.65 ± 2.35 b</td>
<td>73.48 ± 0.93 c</td>
<td>3.73 ± 0.20 d</td>
<td>114.04 ± 3.31 d</td>
<td>1.04 ± 0.15 cd</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>115.00 ± 4.87 bd</td>
<td>-29.28 ± 0.88 b</td>
<td>-2.82 ± 0.43 b</td>
<td>42.54 ± 1.54 a</td>
<td>73.60 ± 0.42 c</td>
<td>3.94 ± 0.21 d</td>
<td>114.82 ± 0.43 d</td>
<td>1.13 ± 0.11 bd</td>
</tr>
</tbody>
</table>

**Table 4.4.** The details of the water, amylopectin, and amylose melts on the DSC curve for soy and wheat dough.
<table>
<thead>
<tr>
<th>Bread Type</th>
<th>Frozen Storage Time</th>
<th>Total Water Content (%)</th>
<th>Peak Water Loss (°C)</th>
<th>FW (%)</th>
<th>UFW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>0 wks</td>
<td>49.1 ± 1.3 a</td>
<td>68.5 ± 3.3 a</td>
<td>40.5 ± 6.9 a</td>
<td>8.5 ± 6.9 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>48.9 ± 0.9 a</td>
<td>73.0 ± 3.8 ab</td>
<td>31.8 ± 5.1 ab</td>
<td>17.1 ± 5.1 ab</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>49.1 ± 0.6 a</td>
<td>74.1 ± 3.6 b</td>
<td>28.1 ± 3.8 b</td>
<td>21.1 ± 3.8 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>0 wks</td>
<td>42.3 ± 1.8 b</td>
<td>64.7 ± 2.7 c</td>
<td>28.8 ± 5.5 b</td>
<td>13.5 ± 5.5 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>41.9 ± 0.4 b</td>
<td>65.0 ± 5.4 c</td>
<td>29.8 ± 3.9 b</td>
<td>12.1 ± 3.9 a</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>42.1 ± 1.0 b</td>
<td>66.0 ± 2.4 c</td>
<td>24.5 ± 6.0 b</td>
<td>17.6 ± 6.0 b</td>
</tr>
</tbody>
</table>

**Table 4.5.** The moisture content, peak of the derivative weight loss, and percent “freezable” (FW) and “unfreezable” (UFW) water in soy and wheat bread baked from fresh dough or dough that was frozen for 2 or 4 wks. The percentages represent the percent of the total weight of the sample. Different lowercase letters denote statistical significance between time points for the given type of bread or between different types of bread at the same time point.
<table>
<thead>
<tr>
<th>Bread Type</th>
<th>Frozen Storage Time</th>
<th>$\Delta H$ of Water Melt (J/g)</th>
<th>$T_{\text{onset}}$ (°C)</th>
<th>$T_{\text{peak}}$ (°C)</th>
<th>$T_{\text{range}}$ (°)</th>
<th>Temp. of Amylose Melt (°C)</th>
<th>$\Delta H$ of Amylose Melt (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>0 wks</td>
<td>135.1 ± 23.1 a</td>
<td>-29.7 ± 1.1 a</td>
<td>-3.48 ± 1.2 a</td>
<td>42.2 ± 1.3 a</td>
<td>109.6 ± 1.5 a</td>
<td>0.79 ± 0.21 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>106.0 ± 17.1 b</td>
<td>-28.1 ± 0.7 b</td>
<td>-0.59 ± 1.6 b</td>
<td>41.7 ± 1.9 a</td>
<td>111.8 ± 0.9 b</td>
<td>0.67 ± 0.07 ab</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>93.5 ± 12.5 c</td>
<td>-27.5 ± 0.9 b</td>
<td>-0.63 ± 1.3 b</td>
<td>41.9 ± 1.8 a</td>
<td>112.2 ± 0.9 b</td>
<td>0.58 ± 0.09 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>0 wks</td>
<td>95.9 ± 18.3 bc</td>
<td>-24.7 ± 2.9 c</td>
<td>-3.43 ± 1.8 a</td>
<td>35.8 ± 3.5 b</td>
<td>115.9 ± 1.8 c</td>
<td>0.76 ± 0.18 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>99.3 ± 13.0 bc</td>
<td>-24.0 ± 2.4 c</td>
<td>-3.72 ± 1.5 a</td>
<td>36.3 ± 2.0 b</td>
<td>113.7 ± 1.3 d</td>
<td>1.21 ± 0.45 c</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>81.6 ± 20.0 d</td>
<td>-22.9 ± 2.0 d</td>
<td>-2.90 ± 1.9 a</td>
<td>34.9 ± 1.7 b</td>
<td>114.3 ± 1.3 d</td>
<td>0.66 ± 0.31 a</td>
</tr>
</tbody>
</table>

**Table 4.6.** DSC was used in bread to observe the temperature and the $\Delta H$ associated with the solid to liquid phase transitions of water and amylose. Different lowercase letters signify statistical significance between time points for the given type of bread or between different types of bread at the same time point.
<table>
<thead>
<tr>
<th>Bread Type</th>
<th>Frozen Storage Time</th>
<th>Loaf Density (g/cc)</th>
<th>Hardness (N)</th>
<th>Springiness (mm)</th>
<th>Cohesiveness (no unit)</th>
<th>Chewiness (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy</td>
<td>0 wks</td>
<td>0.53 ± 0.03 a</td>
<td>6.26 ± 1.73 a</td>
<td>7.43 ± 0.51 a</td>
<td>0.61 ± 0.03 a</td>
<td>36.45 ± 9.74 a</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>0.62 ± 0.03 b</td>
<td>7.05 ± 1.29 b</td>
<td>6.61 ± 0.49 b</td>
<td>0.63 ± 0.02 a</td>
<td>42.55 ± 7.26 b</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>0.63 ± 0.01 b</td>
<td>6.76 ± 0.95 ab</td>
<td>6.70 ± 0.46 b</td>
<td>0.61 ± 0.04 a</td>
<td>39.78 ± 6.14 ab</td>
</tr>
<tr>
<td>Wheat</td>
<td>0 wks</td>
<td>0.42 ± 0.05 c</td>
<td>3.67 ± 1.06 c</td>
<td>7.26 ± 0.31 a</td>
<td>0.49 ± 0.02 b</td>
<td>17.25 ± 4.93 c</td>
</tr>
<tr>
<td></td>
<td>2 wks</td>
<td>0.50 ± 0.01 a</td>
<td>4.35 ± 0.51 cd</td>
<td>6.72 ± 0.32 b</td>
<td>0.46 ± 0.04 c</td>
<td>19.62 ± 3.56 c</td>
</tr>
<tr>
<td></td>
<td>4 wks</td>
<td>0.51 ± 0.01 a</td>
<td>4.76 ± 0.69 d</td>
<td>6.84 ± 0.32 b</td>
<td>0.46 ± 0.02 bc</td>
<td>21.52 ± 3.31 c</td>
</tr>
</tbody>
</table>

**Table 4.7.** Textural properties of bread baked from fresh dough and bread baked from frozen dough.
Chapter 5

The effect of soy addition on the changes of a bread dough model during frozen storage:

A magnetic resonance study

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Abstract

Hygroscopic soy ingredients were hypothesized to slow the rate of water migration in unleavened bread dough during frozen storage. Thawed soy (18% dry weight) and wheat dough samples were assessed using non-destructive nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) for up to 8 weeks frozen storage time. MRI suggested a spatially homogeneous, net increase in proton mobility with frozen storage and, with solution state proton NMR, distinct “free” and “bound” states were discerned. $T_2$ relaxation times of the majority proton population suggested increased mobility with frozen storage time, and statistical difference from the fresh sample was seen later for the soy samples than the wheat samples. As seen by $^{13}$C-solid state NMR, the crystallinity of the starch was not affected by either soy addition or frozen storage. In conclusion, addition of soy to bakery products led to slightly enhanced preservation of “fresh” characteristics of the dough during frozen storage.
5.1. Introduction

Frozen dough comprises an increasing proportion of the bread industry [200]. However, bread made from frozen dough often has less favorable quality characteristics than bread baked from fresh dough [193]. During freezing, water undergoes an energetically favorable migration from the starch and the gluten macromolecular matrix to ice crystals [17], causing the dough matrix to become dehydrated and brittle, eventually causing irreversible damage to the starch granules and separation of the starch granules and gluten [17]. When the dough is thawed, the water redistributes but the gluten and starch granules remain partially rearranged, compromising rise in the loaf during proofing [193]. Therefore, dough formulated to bind water may attenuate these deleterious effects. The hypothesis was that soy, due to the strong water-binding abilities of soy protein [13], would decrease the migration of water during frozen storage thereby resulting in a higher quality frozen dough product.

Magnetic resonance (MR) is a powerful physical technique that offers the ability to observe the liquid-like (ex. water) and the solid-like (ex. starch crystallization) components of dough [201]. Solution state NMR is often performed on low field, table top NMR instruments (0.5 T, 23 MHz proton frequency). However, nuclei respond differently to pulses at high field-strength (ex. 7.05 T, 300 MHz) vs. low field strength allowing the acquisition of additional information about the sample components. Moreover, because of the higher differences in thermal equilibrium populations of the spin states, high field NMR has much higher sensitivity which can be especially useful in the assessment of heterogeneous food samples. The stronger signal may potentially be able to better discern different populations of spins, for example more mobile and less mobile water molecules [202].

In order to probe the properties of water in dough as a function of frozen storage, $T_1$ and $T_2$ relaxation experiments using proton solution state NMR have been used [201]. On a double log scale, as a function of correlation time, $T_1$ relaxation times decrease then increase
in a parabolic fashion (the minimum is present where the Larmor frequency is equal to the correlation time) while $T_2$ times decrease exponentially. Bulk water exhibits a low correlation time and high $T_1$ and $T_2$ relaxation times whereas water in a viscous solution, such as bread dough, generally exhibits lower $T_1$ and $T_2$ relaxation times [201]. This relationship also suggests that increased $T_1$ and $T_2$ times correlate with higher proton mobility. In a frozen dough system, relaxation times were shown to increase with increased frozen storage [55], likely due to the formation of ice crystals during storage, and it is hypothesized that the rate and/or extent of change during frozen storage can be modulated by the hygroscopic properties of soy protein.

To assess water dynamics on a macroscale, MR imaging (MRI) can be used to determine proton density and proton mobility on the millimeter-centimeter scale. MRI has been used successfully by Lodi et al. to characterize the movement of water in bread during ambient storage [32]. Fresh dough is relatively homogenous on a macroscale [203], but consists of microscopic compartments of starch granules, water droplets, and gluten networks, among others [17]. During freezing and under subzero storage conditions, it is energetically favorable for some molecules, especially water, to crystallize [17] and increase the size of their respective compartments. The Lucas group [204] (and references within) has extensively studied the changes in the properties of water during freezing, proofing, and baking using MR with a spatial resolution of approximately 1 mm. With MRI, they have modeled ice crystal formation during freezing; generated maps of ice crystals; monitored heterogeneities that arise during freezing, proofing, and baking; and quantified the number and size of bubble formation during bread preparation. However, it is unknown what effects a hygroscopic ingredient such as soy will have on water properties in fresh or frozen dough.

In addition to the assessment of properties of protons, $^{13}$C solid-state NMR is a useful MR technique to assess crystallinity of starch. In dough, starch granules have been moistened
and are partially gelatinized but, since the dough has not yet been heated, gelatinization is not extensive [205]. Native wheat starch often displays A-type diffraction patterns from x-ray diffraction methods [206]. Little research has been performed on the effect of frozen storage on native starch crystallites and, since the starch fraction of soy dough is diluted compared to wheat dough, it is of interest how the wheat starch properties are affected by soy addition in fresh or frozen dough.

The purpose of this investigation was to characterize the molecular effects of soy addition to frozen dough using MR techniques.

5.2. Materials and Methods

5.2.1. Dough preparation and storage

Soy and wheat dough were prepared based on formulae published by Zhang et al. [28]. Ingredients are shown in Table 5.1. Soy flour and soy milk powder were combined in an approximate 3:1 ratio so that soy ingredients comprised 18.0% of the dry weight [12]. Yeast was omitted to prevent changes in the matrix during analysis due to gas production. The ingredients were combined with approximately 45.3% water for the soy dough and 37.7% water for the wheat dough (because soy dough requires more water for adequate hydration [28]) and shortening was added last. For “fresh dough” results, analyses were performed within 5 hrs of preparation of the dough. For “frozen dough”, 41.25 mL glass vials for NMR or 22.0 mL glass vials for MRI were filled with the dough sample. Jars were capped and wrapped in parafilm to prevent water evaporation. The dough was flash frozen at -40°C for 24 hrs [41] and then transferred to -20°C for the remainder of the frozen storage period (2-8 wks total). Samples were thawed at ambient temperature the day of analysis. For NMR, dough was transferred to NMR tubes; for MRI, the samples were analyzed directly.
5.2.2. \( T_1 \) and \( T_2 \) relaxation with solution state NMR

About 4 cm of dough was placed near the bottom of a 5-mm disposable NMR tube. Spin-lattice (\( T_1 \)) and spin-spin (\( T_2 \)) relaxation experiments were performed at room temperature on a 7.05 T (300 MHz proton resonance) Bruker DMX NMR spectrometer (Bruker Biospin, Rhinstetten, Germany) using the saturation recovery pulse sequence and the CPMG pulse sequence, respectively. The intensity of the \(^1\text{H}\) peak for water (ca. 4.5-5.0 ppm [32]) vs. delay time was used to fit an exponential rise to maximum equation to attain \( T_1 \) relaxation times. The relaxation curves fit best to a double exponential equation (1), thereby distinguishing two distinct \(^1\text{H}\) water populations (attributed to different water populations [207]).

\[
I(\tau) = I(0) + P_1 \times (1 - e^{-\tau/T_{1(1)}}) + P_2 \times (1 - e^{-\tau/T_{1(2)}}) \tag{5.1}
\]

\( I(\tau) \) is the intensity of the peak at time delay \( \tau \), \( P_1 \) and \( P_2 \) are proportionality constants of populations 1 and 2, and \( T_{1(1)} \) and \( T_{1(2)} \) are the respective relaxation times for populations 1 and 2. \( T_2 \) relaxation data were collected on the same dough samples and analyzed using equation 2.

\[
I(\tau) = I(0) + P_1 \times e^{-\tau/T_{2(1)}} + P_2 \times e^{-\tau/T_{2(2)}} \tag{5.2}
\]

Relaxation times were acquired in duplicate.

5.2.3. Magnetic resonance imaging (MRI)

MRI experiments on dough were performed on a 4.7 T/40 cm magnet controlled by a Bruker Avance Console (Bruker Biospin, Billerica, MA) at ambient temperature. The instrument was equipped with a 260 mm-inner diameter gradient coil and a 200 mm-inner diameter proton volume radiofrequency coil. Within the coil, one soy dough sample and one wheat dough sample were placed beside a phantom of \( \text{D}_2\text{O}/\text{H}_2\text{O} \) as a reference for intensity. The ratio of \( \text{D}_2\text{O}/\text{H}_2\text{O} \) was determined by adding just enough \( \text{H}_2\text{O} \) to achieve a comparable
signal to the average sample. MRIcro (version 1.40, © Chris Rorden) was used to observe images and analyze $T_2$ times and standard deviations. Additionally, signal was analyzed separately and fit to double exponential equations using Matlab (R2009b, MathWorks, Inc., Natick, MA), however the signal to noise ratio was not sufficient to resolve distinct populations of $T_2$ values.

5.2.4. $^{13}$C Solid State NMR

Thawed dough was packed into a 4 mm zirconia MAS rotor (Brüker Biospin, Billerica, MA) to fill the space. $^{13}$C-solid state NMR spectra were acquired with a 7.05 T (75.47 MHz carbon resonance, 300 MHz proton resonance) NMR spectrometer with magic angle spinning at room temperature (22-24°C). The sample was spun at 5000 Hz. The $^{13}$C CP/MAS NMR spectra were obtained with a cross-polarized (CP) pulse sequence and a repetition time of 2 sec. A total of 32,768 scans were performed.

5.2.5. Statistical analysis

For $T_1$ and $T_2$ solution state relaxation data and proton intensity and $T_2$ relaxation times collected with MRI, an analysis was performed to fit the model $Y = \text{dough} + \text{time} + \text{dough} \times \text{time}$, where dough = wheat or soy, time = the length of frozen storage time in weeks, and dough$\times$time = the interaction between type of dough and length of frozen storage time. Least-square means for the dough$\times$time interaction were calculated for each analysis using SAS 9.1 (SAS Institute, Cary NC) and $p < 0.05$ was deemed statistically significant. Values are reported as mean ± standard deviation throughout the test.
5.3. Results

5.3.1. T$_1$ relaxation of wheat and soy dough

High field $^1$H NMR spectroscopy was used to assess longitudinal relaxation parameters in wheat and soy dough. A one-dimensional $^1$H spectrum showed one major peak near 4.5-5.0 ppm, assigned to water, and two minor peaks up field, assigned to carbohydrates and lipids (data not shown) [32]. The intensity of the water peak was fit to a double exponential curve, revealing two distinct proton populations in both types of dough. For both soy and wheat dough, population 1 ($P_1$) represented approximately 94% of the proton population, and also had relaxation times one order of magnitude longer than population 2 ($P_2$, Table 5.2). Fresh wheat dough exhibited $T_1(P_1)$ relaxation times approximately 100 msec longer than fresh soy dough (485.1 ± 11.0 msec for wheat vs. 386.1 ± 4.3 msec for soy). For both dough formulations, $T_1(P_1)$ shortened approximately 10% at 2 wks frozen storage time ($p < 0.05$) and then remained relatively stable through 8 wks of frozen storage ($p > 0.05$). The longitudinal relaxation times associated with $P_2$ did not change significantly with frozen storage time.

5.3.2. T$_2$ relaxation of wheat and soy dough

Spin-spin relaxation times were assessed for both soy and wheat dough during 8 wks of frozen storage. As in the $T_1$ experiments, 2 populations were defined, $P_1$ and $P_2$. These labels were arbitrarily assigned so that $P_1$ comprises the larger population in dough, but these labels do not necessarily correspond to the same protons in $P_1$ and $P_2$ from the $T_1$ experiments. In the $T_2$ relaxation experiments, the two populations were about equally represented in fresh dough (about 50% each) for both soy and wheat dough (Table 5.3). With frozen storage, however, the population distribution shifted so that $P_1$ represented approximately 90% of the total proton population after 2 wks in wheat dough and 4 wks in
soy dough (Table 5.3). $T_2(P_1)$ relaxation times ranged from about 1.4 – 4.2 msec while $T_2(P_2)$ times ranged from about 9 – 31 msec. After frozen storage, $T_2(P_1)$ relaxation times tended to become longer in both wheat and soy varieties. On the other hand, $T_2(P_2)$ values tended to become shorter after frozen storage. Notably, unlike the wheat dough, the soy dough that was frozen for 2 wks appeared to retain the properties of fresh dough before exhibiting a shift in relaxation times.

### 5.3.3 MRI of wheat and soy dough

While solution state NMR provides an average of all of the components in the sample, MRI allows spatial resolution of these on a mm-cm scale. Figure 5.1 shows example proton density maps for samples of fresh soy and wheat dough. The proton density is not evenly distributed on the scale of the spatial voxel (about 500 µm × 500 µm in Figure 5.1), as indicated by the granular appearance of the images. On a larger scale, however, assessment of random areas approximately 1 cm in diameter within each image did not reveal differences between the interior and the perimeter of the samples, nor was mm-cm scale water migration (proton intensity change) observed with frozen storage. Different samples were assessed at each time point to avoid effects of freeze-thaw cycles, so it was not possible to perform point-by-point comparisons. However, small pockets of high or low density did not appear to change size with frozen storage. There was no effect of treatment or time between average proton intensities ($p > 0.05$).

Average $T_2$ values were determined for each voxel in each sample. In agreement with the solution state NMR data, average $T_2$ values for fresh soy dough were shorter than those for wheat dough. Also, as frozen storage time increased, $T_2$ times increased (Figure 5.2), suggesting higher proton (water) mobility. Due to the high number of voxels, all data sets were statistically significant from one another ($p < 0.001$). However, the difference between
the average $T_2$ time for fresh and 2 wk stored dough was much higher for wheat dough (0.55 msec) than soy dough (0.16 msec), consistent with the solution state $T_2$ data. The distribution in $T_2$ times also increased with frozen storage time, especially for the soy dough (0.60 msec fresh to 0.65 msec at 2 wks and 0.95 msec at 4 wks).

5.3.4. $^{13}$C CP-MAS solid state NMR

In order to probe changes in starch crystallization during the frozen storage period of the dough sample, $^{13}$C CP-MAS solid state NMR was employed. Peaks were present predominately for carbon atoms in crystalline starch (Figure 5.3), as assigned by Gidley & Bociek [208], and a small protein signal at 170-180 ppm in the soy samples was observed [209]. The C-1 set of peaks for fresh and frozen soy and wheat dough showed 4 smaller peaks within, suggesting an A-type crystallization pattern (the more densely packed structure compared to B-type) [208]. As frozen storage time increased, neither the chemical shift nor lineshape changed dramatically, indicating that there was little to no rearrangement of helix packing during frozen storage. Moreover, no change was observed in either the chemical shift or the lineshape for the soy protein peak with frozen storage time.

5.4. Discussion

The possibility of soy ingredients to modulate water migration during frozen storage was assessed using MR techniques. Analysis of water in bread dough is somewhat difficult due to its complex, heterogeneous structure. It is generally believed that water is present in three separate yet loosely defined compartments within dough: 1) in small water pools displaying properties similar to bulk water, 2) tightly bound to starch and protein molecules, thereby non-exchangeable and “unfreezable”, and 3) at the interface of the liquid droplets, thereby transiently being ordered by macromolecular structures but also participating in
exchange and cross-relaxation processes [202]. However, even within these populations, there is a large variance in properties of the water and significant changes can occur over time since dough is not at equilibrium [202,210]. Depending on both the sample and the analysis technique, it is sometimes even difficult to discern these three populations [202]. In shelf life studies, however, it is not practical or necessary to identify or describe all of the populations of water, but to quantify the extent by which the system changes with storage.

$T_1$ relaxation analysis with solution state $^1$H NMR was used to assess the spin-lattice relaxation properties of the protons in the dough which primarily depict water properties [202]. $T_1$ data revealed two distinct populations of water in a ratio of approximately 10:1 (Table 5.2). The population distribution was stable with frozen storage time for both soy and wheat dough varieties (Table 5.2). Although the observed $T_1$ times may be slightly less than actual $T_1$ spin-lattice values due to cross-relaxation behaviors [211], soy dough exhibited shorter $T_1$ times for the majority population at every time point, suggesting that the water molecules were more translationally and/or rotationally hindered than those in the wheat dough [207]. Literature on spin-lattice relaxation experiments in dough performed using high field NMR (> 7.05 T, 300 MHz $^1$H frequency) is sparse and, due to the strong dependence of $T_1$ relaxation times on field strength, it is not appropriate to compare results to data collected at low field strength (ca. 0.5 T, 23 MHz $^1$H frequency). With that said, D’Avignon et al. [212] has performed $^2$H spin-lattice relaxation experiments on dough at high field strength (7.05 T). They found that increased water content of dough from 0.1-0.2 g deuterated water ($D_2O$) per gram of dry solid (flour) does not have an effect on $T_1$ time but further increases in $D_2O$ amount (0.2-1.2 g $D_2O$ per gram of dry solid) led to a longer relaxation time. This suggests that the majority of the water in a typical dough system (approximately 40-50% water) is in the extreme narrowing limit ($\omega \tau_c << 1$ where $\omega$ is the Larmor frequency and $\tau_c$ is the correlation time) and, consequently, protons that exhibit higher $T_1$ values have properties
that more closely resemble those of bulk water. The minority populations in the soy and wheat dough samples showed no differences in $T_1$ relaxation times between one another or changes with frozen storage time (Table 5.2). $T_1$ relaxation times for the majority population were all shorter in stored dough compared to the fresh samples, suggesting that rearrangement of helices akin to staling occurred upon freezing [213]. Since there was not a discernible trend in $T_1$ relaxation times with increased frozen storage, it is thought that degradation of the matrix as a result of water migration was not extensive in either sample during this period of frozen storage.

Spin-spin relaxation times were also probed with $^1$H solution state NMR in order to further describe changes in proton (water) mobility with frozen storage time. Again, two populations were defined, $P_1$ and $P_2$, with approximately equal representation in both soy and wheat fresh dough (Table 5.3). Because of the discrepancy in population distribution, it is hypothesized that the populations that were defined in the $T_1$ and $T_2$ relaxation experiments are not the same populations. (Strong exchange and cross-relaxation properties can affect relaxation properties differently [211], so it may be best to analyze these data separately). With increasing frozen storage time, water appeared to migrate from $P_2$ (the higher mobility fraction) to $P_1$ (the lower mobility fraction), so that $P_1$ contributed to approximately 90% of the signal in frozen dough (Table 5.3). This implies that the water became less mobile with frozen storage time. However, when the less mobile fraction increased, it exhibited properties resembling bulk water (longer $T_2$ times) and, accordingly, the more mobile fraction got smaller and exhibited shorter $T_2$ times.

$T_2$ values are less dependent on field strength than $T_1$ values, allowing more appropriate comparisons to values acquired by other authors at low field strength (typically 23 MHz). During frozen storage, water phase-separates from the gluten and starch matrix to form ice crystals. Although water redistributes upon thawing, it is not expected that the water
will reoccupy congruent configurations because of permanent damage to the matrix [17,55]. Esselink et al. [55] and Yi, Johnson, & Kerr [57] have also described an increase in $T_2$ values with frozen storage time. Our results agree with those of Yi et al. [57] who described dough samples with two major populations of water; a major population (85-88%) with a lower $T_2$ relaxation time than the minor population. However, they did not report a shift in populations and saw an increase in $T_2$ times with only 1 day of frozen storage [57]. The longer $T_2$ times were attributed to water that had leached from the macromolecular matrix into ice crystals during frozen storage, thereby existing in larger water droplets and more resembling bulk water. Differences in formulation (higher water content, inclusion of yeast, frozen storage temperature, etc.) may have contributed to differences in these observations [55]. Moreover, sample preparation (preparing samples while still frozen vs. after thawing) could also drastically affect the properties of water. When comparing wheat and soy formulations, the soy dough exhibited a slower rate of change of $T_2$ relaxation values with changes in statistical significance between 2 and 4 wks frozen storage vs. 0 to 2 wks in the wheat dough. This suggests that, like in baked bread [39], soy binds water more strongly and slows water migration during storage.

Data collected from spatial MRI experiments revealed proton density maps showing water was distributed in small pockets (on the scale of the observed voxels, 500 µm) but evenly throughout the sample slice (Figure 5.1). This has been observed previously in fresh bread dough [55,203]. Water did not appear to migrate on a mm-cm spatial scale in dough after 2 or 4 wks frozen storage nor, qualitatively, did the water pockets appear to increase in size, as was observed in Esselink et al. [55]. The dough prepared in Esselink et al. [55] was yeasted and therefore had large air cells which affect the properties of water and their changes during freezing, frozen storage, and thawing. The dough used in our experiment was nonyeasted and thus did not contain different compartments on a macroscale at the onset of
freezing, so it was therefore expected to not see mm-cm water migration. Few studies have been performed on water migration during frozen storage of fully- or partially-baked bread, but because of the water concentration differential between the crumb and the crust, the components of baked bread can freeze at different rates and cause “shelling” (separation of the crumb from the crust) or other structural deterioration [214]. Moreover, akin to the staling process, water migration can occur from the crumb to the crust especially when the dough is in between -7°C and 4°C during freezing and thawing [215]. This minimizes the distinction between bread crumb and crust and may potentially decrease consumer acceptability [215]. Freezing of unbaked or partially baked dough possibly prevents macroscale migration of water during frozen storage.

$T_2$ relaxation data collected with MRI essentially provides thousands of replicates within each sample. These data show that the average and standard deviation of $T_2$ relaxation times increased with increasing frozen storage (Figure 5.2). Consistent with the solution state NMR data, the wheat dough exhibited higher $T_2$ than the soy dough, and $T_2$ increased with frozen storage time. This corroborates the notion that soy binds water molecules strongly, thereby lessening mobility, and that the mobility of the water increases with frozen storage time. In addition, MRI revealed that the distribution of $T_2$ times became broader (Figure 5.2) with frozen storage time, especially in the soy dough, implying that the protons are occupying a larger number of heterogeneous sites within the dough matrix.

In addition to assessing water distribution, starch mobility and crystallization were probed qualitatively using solid state $^{13}$C CP-MAS. The C-1 peak represents the type of starch crystallinity and showed 4 smaller peaks within the larger peak. The two smaller peaks are indicative of B-type crystallization while 3 usually indicates A-type crystallization [208]. Raw dough possesses native starch (swollen starch granules but not gelatinized starch) and native wheat starch usually has an A-type crystallization pattern [216]. Four peaks were
present in all dough samples in our experiments, regardless of formulation (both wheat and soy dough contained wheat starch) or frozen storage (Figure 5.3). Over 36,000 scans were performed leading to unprecedented resolution, potentially explaining the fourth peak that was observed in addition to the canonical 3 peaks associated with A-type crystallization. The process of freezing, frozen storage, and thawing did not disrupt the crystallization pattern, suggesting that soy addition will not impact the functionality of the starch.

During freezing, the decrease in temperature leads to a glass transition of the starch between 0°C and -20°C, resulting in a conversion from an amorphous (rubbery) to a glassy state [217]. In a $^{13}$C CP-MAS experiment performed at low temperatures on a starch gel by Vodovotz et al. [217], an increase in peak intensity due to immobilization of the helices, a reduction of mobility of the macromolecular structures, and/or a simple rearrangement of the matrix led to more efficient cross polarization. In many dough and starch gel studies, starch has been partially gelatinized and the starch granules retain some structure. Therefore, it is not expected for much further crystallization of starch to take place during frozen storage. Moreover, phase separations (ex. starch granules from gluten or macromolecules from water) can contribute to large changes in the physical and molecular properties of the starch in dough, especially over the time scale of weeks [17]. Some changes are reversible, but ice crystal formation can also permanently damage starch granules [17]. When the dough is thawed, starch helix structure and mobility is partially, but not completely, restored. Additional quantitative studies that assess peak intensity with frozen storage time could help shed light on changes in the degree of starch crystallization (native starch is approximately 30% crystalline, [216]) and/or breakage of starch crystallites during frozen storage.

Based on both the water and the starch data, it appears that permanent changes are occurring in the wheat dough matrix that are being either retarded or prevented by modulation of the water fraction by soy addition.
5.4.5. Conclusions

Two different states of water were characterized in fresh, non-yeasted dough using solution state NMR, attributed loosely to more mobile and less mobile water, at an approximate 10:1 ratio. Both solution state NMR and MRI relaxation data revealed that the protons in soy dough were less mobile than those in wheat dough, corroborating the hypothesis that soy binds water tightly in the dough matrix. Also, there was no water migration observed on a mm-cm scale of the non-yeasted dough during frozen storage. $^{13}$C CP-MAS showed that the starch within the dough resembled that of native starch from previous studies and neither soy addition nor frozen storage affected the integrity of the crystalline structures. Hence, when comparing our “functional” soy dough to common wheat dough, it appeared that the addition of soy slightly improved the preservation of “fresh” characteristics.

5.5. Acknowledgments

We would like to thank Dr. Amir Abduljalil for help with acquisition of the MRI data and Dr. Tanya Young for help with acquisition of the solid state NMR data. We would also like to thank Dr. Ian Kleckner, Dr. Robert Curley Jr., and Dr. Joseph Sachleben for thoughtful discussions in the analysis of the data.
Figure 5.1. Example MRI images of soy (S) and wheat (W) dough with the phantom (P) sample (H₂O/D₂O) as an intensity reference.
Figure 5.2. Histograms showing average $T_2$ values for soy (black) and wheat (gray) dough samples, acquired as fresh dough (solid line), 2 wks frozen storage (dashed line), or 4 wks frozen storage (dotted line).
Figure 5.3. Example solid state $^{13}$C-CP-MAS NMR spectra for wheat (left) and soy (right) dough. Peak assignments by Gidley & Bociek [208].
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Source</th>
<th>Wheat</th>
<th>Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring wheat flour</td>
<td>Magnifico, 13.0% protein, ConAgra Mills, Omaha, NE</td>
<td>897.31 g</td>
<td>717.27 g</td>
</tr>
<tr>
<td>Non-toasted, defatted</td>
<td>Archer Daniels Mills, Decatur, IL</td>
<td>0 g</td>
<td>135.03 g</td>
</tr>
<tr>
<td>soy flour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soymilk powder</td>
<td>Devansoy, Inc., Carroll, IA</td>
<td>0 g</td>
<td>45.01 g</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>Hodgen Mill, Effingham, IL</td>
<td>14.40 g</td>
<td>14.40 g</td>
</tr>
<tr>
<td>Dough conditioner</td>
<td>The Prepared Pantry, Rigby, ID</td>
<td>1.87 g</td>
<td>1.87 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>US Food Service, Columbia, MD</td>
<td>14.40 g</td>
<td>14.40 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>US Food Service, Columbia, MD</td>
<td>36.01 g</td>
<td>36.01 g</td>
</tr>
<tr>
<td>Vegetable shortening</td>
<td>Crisco®, J. M. Smucker Co., Orrville, OH</td>
<td>36.01 g</td>
<td>36.01 g</td>
</tr>
</tbody>
</table>

**Table 5.1.** Ingredients used in the soy and wheat formulations based on 1000 g batches.
<table>
<thead>
<tr>
<th>Time (wks)</th>
<th>Wheat</th>
<th></th>
<th>Wheat</th>
<th></th>
<th>Wheat</th>
<th></th>
<th>Soy</th>
<th></th>
<th>Soy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$P_1$ (percent of total)</td>
<td>$P_2$ (percent of total)</td>
<td>$T_1(P_1)$ (msec) (liquid like)</td>
<td>$T_1(P_2)$ (msec) (solid like)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>94.2 ± 0.3% a</td>
<td>5.8 ± 0.3% a</td>
<td>485.1 ± 11.0 a</td>
<td>19.4 ± 0.1 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>93.3 ± 0.1% a</td>
<td>6.7 ± 0.1% a</td>
<td>437.7 ± 2.6 b</td>
<td>20.4 ± 2.2 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>93.8 ± 0.2% a</td>
<td>6.2 ± 0.2% a</td>
<td>447.7 ± 9.4 b</td>
<td>20.2 ± 0.2 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>93.5 ± 0.3% a</td>
<td>6.5 ± 0.3% a</td>
<td>432.8 ± 7.9 b</td>
<td>21.2 ± 1.6 a</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>93.5 ± 0.1% a</td>
<td>6.5 ± 0.1% a</td>
<td>432.4 ± 5.6 b</td>
<td>19.8 ± 1.5 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.3 ± 0.2% a</td>
<td>5.7 ± 0.2% a</td>
<td>386.1 ± 4.3 c</td>
<td>20.6 ± 3.9 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>94.0 ± 0.1% a</td>
<td>6.0 ± 0.1% a</td>
<td>353.9 ± 5.1 d</td>
<td>17.9 ± 1.2 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>93.8 ± 0.1% a</td>
<td>6.2 ± 0.1% a</td>
<td>344.3 ± 8.1 d</td>
<td>20.0 ± 2.7 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>94.1 ± 0.0% a</td>
<td>5.9 ± 0.0% a</td>
<td>346.1 ± 3.1 d</td>
<td>19.8 ± 1.2 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>94.0 ± 0.3% a</td>
<td>6.0 ± 0.3% a</td>
<td>356.7 ± 3.6 d</td>
<td>20.5 ± 3.2 a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.2.** The proportion of protons in populations $P_1$ and $P_2$ (percentage) with their respective $T_1$ values. Time = 0 represents fresh dough samples and other time points indicate weeks of frozen storage. Different letters represent statistical significance within each column.
<table>
<thead>
<tr>
<th></th>
<th>Time (wks)</th>
<th>$P_1$ (percent of total)</th>
<th>$P_2$ (percent of total)</th>
<th>$T_2(P_1)$ (msec) (solid like)</th>
<th>$T_2(P_2)$ (msec) (liquid like)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>0</td>
<td>48.8 ± 1.5% a</td>
<td>51.2 ± 1.5% a</td>
<td>1.4 ± 0.1 a</td>
<td>30.6 ± 0.5 a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>90.0 ± 1.9% b</td>
<td>10.0 ± 1.9% b</td>
<td>2.7 ± 0.6 ab</td>
<td>8.8 ± 3.8 b</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>92.7 ± 0.9% b</td>
<td>7.3 ± 0.9% b</td>
<td>4.2 ± 0.0 b</td>
<td>13.8 ± 2.2 b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>88.3 ± 2.9% b</td>
<td>11.7 ± 2.9% b</td>
<td>3.9 ± 0.2 b</td>
<td>13.2 ± 2.1 b</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>91.1 ± 2.5% b</td>
<td>8.9 ± 2.5% b</td>
<td>4.1 ± 0.1 b</td>
<td>16.5 ± 3.5 b</td>
</tr>
<tr>
<td>Soy</td>
<td>0</td>
<td>52.1 ± 0.6% a</td>
<td>47.9 ± 0.6% a</td>
<td>1.5 ± 0.1 a</td>
<td>29.2 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50.4 ± 1.5% a</td>
<td>49.6 ± 1.5% a</td>
<td>1.5 ± 0.1 a</td>
<td>28.5 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>93.3 ± 3.1% b</td>
<td>6.7 ± 3.1% b</td>
<td>3.7 ± 0.2 b</td>
<td>16.6 ± 4.5 b</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>94.7 ± 3.7% b</td>
<td>5.3 ± 3.7% b</td>
<td>3.8 ± 0.2 b</td>
<td>17.2 ± 8.0 b</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>86.1 ± 1.9% b</td>
<td>13.9 ± 1.9% b</td>
<td>3.5 ± 0.1 b</td>
<td>10.8 ± 0.7 b</td>
</tr>
</tbody>
</table>

**Table 5.3.** $T_2$ relaxation data from soy and wheat dough frozen for 0-8 wks. A double exponential fit revealed 2 distinct populations. Time = 0 represents fresh dough samples and other time points indicate weeks of frozen storage. Different letters represent statistical significance within each column.
Abstract

The consumption of nutrient-poor snack foods in Western diets is thought to be contributing to the increasing prevalence of obesity and diabetes. Soy offers unique potential to provide high quality protein, dietary fiber, and phytochemicals to snack foods to produce a more healthful nutritional profile. In this study, 27.3% of wheat flour was replaced with soy ingredients in a soft pretzel and evaluated for impact on satiety, glycemic index (GI), and insulinemic index (II). We first tested the soy pretzel for consumer acceptability by 51 untrained sensory panelists on a 9-point hedonic scale. Second, in a crossover trial, 20 healthy adults consumed soy and traditional pretzels (1000 kJ or 239 kcal each) after an overnight fast. They reported their levels of satiety on a 10 cm visual analog scale (VAS) for 2 hrs postprandially. Third, 12 healthy, non-diabetic subjects consumed soy or traditional pretzels (50 ± 2 g available carbohydrates) to determine the GI and II of both products. Blood glucose and insulin responses were monitored for 2 hrs after consumption and compared to a glucose reference. It was found that a consumer-acceptable soy soft pretzel had a lower mean (±SD) GI than its traditional counterpart: 39.1 (±20.4) for soy and 66.4 (±15.3) for wheat, (p=0.002).
However, soy addition did not statistically affect II ($p=0.15$), or satiety ($p=0.91$). In conclusion, a nutrient-dense soy pretzel formulation with 27.3% of wheat flour replaced by soy ingredients had attenuated postprandial glycemia without significantly affecting insulinemia or satiety in healthy adults.

6.1. Introduction

Snacks are defined as foods and beverages consumed at occasions other than meals. People in the United States are consuming up to 24% of their calories from snacks on average, a significant increase over the last few decades [218]. The increase in food consumption frequency without compensatory energy reduction at each eating occasion may be contributing to the incidence of obesity and type 2 diabetes [3]. Moreover, current snack food choices made by consumers tend to be high in fats and added sugars, contain highly processed carbohydrates that tend to increase blood glucose levels, and contain few essential nutrients or health-promoting phytochemicals [218]. Habitual consumption of foods with these properties can additionally increase risk for nutrient deficiency or impact risk of chronic diseases of aging such as cardiovascular disease and type 2 diabetes [7]. Therefore, snack foods offer an opportunity to include nutritious ingredients to potentially impact the quality of the typical American diet.

Soy incorporation into snack foods can provide nutritional benefits such as high quality protein, fiber, and various micronutrients and phytochemicals [7,28]. It has been shown that soy products may enhance satiety [8,9] which may reduce energy intake and risk of obesity [10]. In addition, diets rich in soy protein may contribute to a lower risk of cardiovascular disease [7,219]. Soy is the only non-animal food source that provides all 20 amino acids [220] while remaining low in saturated fat and cholesterol. Soy flour may contain up to 17% dietary fiber including both soluble and insoluble types of fiber. These
types of fiber may have a beneficial impact upon total and low density lipoprotein (LDL) cholesterol [219,221]. Additionally, soy phytochemicals are proposed to potentially reduce the risk of several cancers [19,130].

Grain-based snacks such as crackers, pretzels, and other bakery products are popular and offer a promising matrix for the delivery of soy. However, soy addition poses challenges for yeast-leavened bakery products [18,28,38,39]. In bread, soy protein strongly binds water and dilutes the gluten matrix, decreasing loaf volume [35]. However, baked snack foods such as pretzels (soft and hard), breadsticks, and crackers have a denser matrix which can accommodate increased soy and therefore provide a more promising, consumer-acceptable delivery system.

The glycemic index (GI) of a food is defined as the area under the curve of glycemia vs. time (2 hrs) immediately following consumption of 50 g available carbohydrates (total carbohydrates minus fiber) from the test food, compared to 50 g pure glucose [89]. The insulinemic index (II) is acquired and assessed with a protocol analogous to that of the GI and is used to compared postprandial insulinemia [114]. Foods that are composed of sugars and refined grains generally possess high GI values (≥70 with pure glucose = 100) while foods with a lower amount of processed carbohydrates have lower GI values (≤55) [222]. Because habitual consumption of high GI foods has been associated with increased risk for type 2 diabetes [91], coronary heart disease [223], and increased appetite that may contribute to obesity [222], there is a desire to increase the availability of low GI snack foods [224]. The effects of soy addition on satiety, GI, and II in baked products has not been reported.

The purpose of this investigation was to evaluate the acceptability of a soft pretzel formulated with 27.3% soy ingredients (dry weight) and determine the GI, II, and satiety of the soy-based soft pretzel compared to a conventional wheat soft pretzel. We hypothesized
that incorporation of soy would lead to an increase in satiety and a decrease in GI and II in a soft pretzel.

6.2. Experimental

Sensory analysis, glycemic/insulinemic, and satiety studies were approved by the Institutional Review Board at The Ohio State University and performed at the Clinical Research Center at The Ohio State University Medical Center, Columbus, Ohio.

6.2.1. Pretzel production

Soy and wheat pretzels were produced using ingredients in Table 6.1. Wheat flour (350 g or 31.7 g/100 g), dough conditioner, wheat gluten, and 325 g water (37.5 g/100 g) were added to a KitchenAid mixer and stirred with a flat beater attachment at a low speed until moistened (about 30-60 sec). To produce the sponge, the dough was proofed at 39°C at 100% humidity for 2 hrs (CM2000 Holding/Proofing Combination Module; InterMetro Industries Corp., Wilkes-Barre, PA). The remaining ingredients except the shortening were added and stirred with the mixer. The flat beater attachment was used with the mixer on a low speed (“2”) until the ingredients were combined (about 1-2 min). The attachment was then replaced with a dough hook and the dough was stirred for approximately 6-8 min more. The shortening was added and the dough was blended until it sheeted (about 5 more min). The dough was rolled into an approximately 60 cm rope and formed into a soft pretzel shape. The pretzel was dipped into 1.0% sodium hydroxide solution (65 ± 5°C) for 45-60 sec and placed on a greased baking sheet (Pam 100% Canola cooking spray; ConAgra Foods, Omaha, NE). The pretzels were proofed for 30 min more then baked at 150°C for 15 min (JA14 Jet-Air oven; Doyon, Linière, QC, Canada).
To determine the energy density, the baked pretzel was dehydrated in a 60°C cabinet (Curtin Matheson, Huston, TX) for 48 hrs and subjected to bomb calorimetry (Parr Adiabatic Calorimeter, Moline, IL). Benzoic acid was used to determine calorimeter efficiency. Amount of fat, carbohydrates, fiber, and protein were calculated based on certificate of analyses and nutrition facts labels.

6.2.2. Sensory analysis

Male and female participants between the ages of 18 and 40 yrs were recruited from the university campus to complete the sensory analysis. Samples were prepared by placing fresh pretzel pieces (less than 24 hr old) into 2 oz plastic portion cup labeled with a random 3-digit number. The participants consumed samples of either a soy or wheat pretzel in random order (counterbalanced) in ambient lighting. The participants reported their level of acceptability on a 9-point hedonic scale with “1”, on the left, being “extremely dislike”, “5” being “neither like nor dislike”, and “9”, on the right, being “extremely like”.

6.2.3. Study 1- Glycemic and insulinemic indices

The GI and II protocols were based on those detailed in Brouns et al. [112]. Pretzel dough totaling 63.8 g (25.0 ± 1.0 g available carbohydrates [112]) was used to prepare pretzels for the GI and II studies. Participants consumed 50.0 ± 2.0 g available carbohydrates from the soft pretzels in the form of 2 soft pretzels. Table 6.2 shows the energy and macronutrient profiles of the dough. Baked soft pretzels were stored at -40°C and thawed at room temperature the day before consumption.

Healthy nonsmokers with a body mass index (BMI) less than 30 and without a history of diabetes, glucose intolerance, gastrointestinal disorders, or wheat or soy allergies were enrolled in the study. After an overnight fast, participants arrived at the Clinical Research
Center (CRC). Their vital signs and weight were recorded and they rested for 30 min. During this time an intravenous catheter was inserted into the medial cubital vein in the left or right arm and, to assure adequate glycogen stores, a dietary record was assessed for intake of at least 150 g of carbohydrates in each of the previous 3 days. At time $t = 0$, a baseline blood sample was drawn and, subsequently, they consumed either a glucose standard drink (Glucola, NERL Diagnostics LLC, East Providence, RI), white bread (Giant Eagle King Size enriched bread, Pittsburgh, PA), a soy pretzel, or a wheat pretzel, each containing 50 g available carbohydrates. The glucose drink was consumed three times— at the first session, the last session, and either session 3 or 4. The solid samples were all consumed once, the order determined by randomized block. Blood samples were drawn at $t = 15, 30, 45, 60, 90,$ and 120 min. At least one week separated each of the 6 visits.

Blood samples were frozen the day of collection and analyzed in a single batch. A YSI 2300 State Plus Glucose and Lactate Analyzer with a sensitivity of 2.5 mg/dl was used to determine glycemia (YSI International, Yellow Springs, OH). Insulin concentrations were determined with an Immulite 1000 chemiluminescence method (Siemens Medical Solutions Diagnostics; Duluth, GA). This assay has a sensitivity of 2 μIU/mL, an intra-assay coefficient of 5.7%, and an inter-assay coefficient of 6.7%. Graphs of glycemia or insulinemia vs. time were generated and the area under the curve (AUC) was calculated for each by measuring the area above the baseline [112]. The average of the AUCs for the three glucose standards was deemed a GI of 100; the same was performed for insulin. The GI and II were reported as the percent AUC as compared to the glucose standard. White bread served as a method validation.
6.2.4. Study 2: Satiety study

The satiety experiment employed a randomized, counterbalanced, cross-over design similar to that in Holt et al. except satiety values were compared between treatments instead of compared to a glucose treatment [10].

Soy dough was weighed to 99.0 g and wheat dough to 98.5 g to obtain soft pretzels with 500 kJ (119.5 kcal) of energy (Table 6.2). Each participant consumed 1000 kJ per session in the form of 2 fresh pretzels (less than 48 hrs old).

Healthy adults age 18-45 yrs with no wheat or soy allergies were eligible. Pregnant women were excluded. Participants were randomly assigned to one of two groups; one group consumed the soy pretzel on day 1 while the other group consumed the wheat pretzel on day 1. After an overnight fast (10-12 hrs) and immediately before breakfast, the participants were instructed to report their state of hunger on a 10 cm visual analog scale (VAS) by placing a vertical line on the scale. The scale was flanked by “Extremely hungry” on the left and “Extremely full” on the right [123]. The participant then consumed either the wheat or the soy pretzel, as instructed by the study designer. The participants were instructed to eat the pretzel as is, without any alterations such as heating or toasting or additions including salt or mustard. Participants reported satiety on congruent VASs at 15 min, 30 min, 1 hr, 1.5 hr, and 2 hr after intake. During the 2 hr period they refrained from eating and drinking with the exception of water with intakes recorded. The participants were allowed to eat or drink *ad libitum* for the rest of the day, although alcohol was discouraged. That night, the participant again fasted for 10-12 hrs and repeated the procedure the following morning for the other type of pretzel (soy or wheat). For analysis, the distance was measured between the left side of the scale and their vertical line. The data were then normalized by setting the baseline measurement at “0 mm” and the resulting values were plotted vs. time. Using the trapezoid rule, the AUC was
calculated for the area above the baseline [112]. Both the satiety declarations at each time point and the AUC of satiety vs. time were used in the statistical assessment.

6.2.5. Statistics

Differences between the soy and wheat soft pretzels were calculated for acceptability, GI, II, satiety values (AUCs), and water consumed during the satiety experiment using a paired, two-tailed, Student’s t-test using Microsoft® Office Excel® 2007 (Microsoft Corp., Redmond, WA). Individual time points of glycemic and insulimemic indices were analyzed using analysis of variance (ANOVA) with the model \( Y = T_{\text{ype}} + \text{Participant} + \text{Time} + T_{\text{ype}} \times \text{Time} \) where \( Y \) = glycemia (mg/dl) or insulinemia (µIU/ml); \( T_{\text{ype}} \) = soy or wheat; Participant is 1-12; and Time = 0, 15, 30, 45, 60, 90, or 120 min. SAS statistical software was utilized (SAS 9.2 TS2M0, SAS Institute, Inc., Cary, NC). Individual satiety scores at each time point were evaluated using Minitab statistical software (Minitab Inc., State College, PA) with the model \( Y = \text{Time} + \text{Type} + \text{Participant} \) where \( Y \) = satiety; Participant = 1-20; and Type and Time are the same as above. Statistical significance was deemed at \( p < 0.05 \).

6.3. Results

No adverse effects were observed for any of the participants during the studies.

6.3.1. Sensory analysis

Fifty-one volunteers (19 male, 32 female) between the ages of 18 and 40 [mean (± SD) age = 26.6 (± 6.3) yrs] completed sensory analysis. The mean (± SD) acceptability of the soy-based soft pretzel was 6.6 (± 1.1) on a 9-point hedonic scale and 6.7 (± 1.2) for the wheat pretzel (\( p = 0.59 \)). These ratings fall between “slightly like” and “moderately like”.
6.3.2. Study 1- Glycemic and insulnemic indices

Thirteen participants were screened and 100% were eligible. One 29 yr old male dropped out before the study began for personal reasons. Six recruits were male and 6 were female; 1 was East Asian and 11 were Caucasian. The age range was 19-33 yrs; mean (± SD) age was 23.8 (± 4.5) yrs.

Blood glucose and insulin values at each of the assessment time points are presented in Figure 6.1 and Table 6.3. The GI was calculated by measuring the AUC of the glycemic response compared to glucose. The mean (± SD) GI for the wheat pretzel was 66.4 (± 15.3) vs. 39.1 (± 20.4) for the soy pretzel (p = 0.002). The AUC for glycemia after soy pretzel consumption was lower than that post-wheat consumption for 10 out of 11 of the participants.

To confirm the reliability of this protocol, the GI for white bread was calculated. The calculated mean (± SD) GI for white bread was 60.4 ± 19.8, which is consistent with average GI (75 ± 2) reported by Atkinson et al. [11].

The AUC for insulnemia vs. time was calculated for the test foods and compared to that of the glucose standard. As a methods confirmation, white bread resulted in a mean (± SD) II of 62.8 (± 18.9), consistent with reported value of 69 (± 24) from Oku et al. [225]. Mean (± SD) II for the wheat pretzel was 79.0 (± 22.6) vs. 75.0 (± 19.6) for the soy pretzel (p = 0.44).

6.3.3. Study 2- Satiety

Twenty participants, 8 males and 12 females, age 20-43 yrs [mean (± SD) age = 25.3 (± 6.4) yrs] were recruited for the satiety study. All screened applicants were eligible and 100% of participants completed the study.

In order to assess the relative satiety levels for each of the pretzels, participants consumed 1000 kJ (239 kcal) of energy from pretzels for breakfast. The mean (± SD) satiety
score (AUC) was 306.2 (± 215.0) cm×min for wheat vs. 311.3 (± 201.0) cm×min for the soy pretzel \( (p = 0.92) \). Moreover, the mean (± SD) amount of water consumed was similar for the 2 hrs after the consumption of both pretzels [342 (± 273) mL for wheat vs. 336 (± 319) mL for soy; \( p=0.91 \)].

6.4. Discussion

Current snack food formulations such as those for cookies, crackers, and hard pretzels tend to have high GIs, yet all but 3 of the 64 soy-based foods from the GI database are “low GI” \((≤55)\) [11,222]. Despite this observation, there are no reported studies that examine the effect of soy substitution alone on the GI or II of bakery products or snack foods. Therefore, a soft pretzel enriched with soy was produced as a model bakery product or snack food.

In this study, consumer acceptability of the soy-based soft pretzel was compared to the traditional soft pretzel. Both pretzels were rated between “slightly like” and “moderately like” indicating that the soy pretzel is a consumer-acceptable substitute for the traditional soft pretzel. In contrast, Tsen reported that bread with more than 11% soy flour was unacceptable according to American bread quality standards, likely due to the denser, moister crumb and darker color [38]. However, Dhingra & Jood formulated acceptable bread with 10% soy in an Indian population [226]. In accord with our results, Sabanis & Tzia utilized soy milk powder to incorporate up to 20% soy ingredients into bread while maintaining favorable sensory attributes in a Greek population [227]. By incorporating approximately 1/3 of the soy ingredient as soy milk powder in this study, textural detriments were partly circumvented while maintaining high protein content. This is likely due to the higher ratio of soluble fiber:insoluble fiber in the soy milk powder compared to defatted soy flour [18]. In addition,
a chewy texture and darker brown color (imparted by the lye bath), while generally not acceptable in bread, is regarded as a positive quality in soft pretzels by consumers.

In the glycemic/insulinemic index study (Study 1), both pretzels were composed of 50 g available carbohydrates. But, because the soy pretzel formulation contained about 20% less starch per gram, each soy pretzel contained an additional 61.3 kcal, 1.8 g fat, 3.3 g fiber, and 9.1 g protein compared to the wheat pretzel (Table 6.2). Despite the elevation in these nutrients, the soy soft pretzel, composed of 27.3% soy ingredients, decreased the GI of a wheat-based soft pretzel. The standard deviations of our indices are higher than those that have generally been reported (Figure 6.1), perhaps because we used venous blood instead of arterial or capillary blood in the interest of participant comfort [112]; we had 100% compliance among those who began the study.

Reduced glycemia can arise from reduced rate of glucose introduction to the blood, increased rate of glucose uptake by tissues, or both [228]. It is possible that at least the former mechanism is involved in the reduced glycemic response to the soy pretzel. The larger amount of total food in the stomach with the soy pretzel may have slowed the transit time from stomach to small intestine, reducing the rate of carbohydrate availability for absorption. The addition of protein has been shown to slow the rate of gastric emptying and, consequently, reduce postprandial glycemia in glucose/gelatin-based beverages [229]. The insulin secretogenetic properties of protein may also have contributed to the lower postprandial glycemic response. Insulinemia has been shown to be higher after a meal with a whey protein pre-load, leading to attenuated post-prandial glycemia [230]. Increased insulin sensitivity has been observed with longer soy interventions [115].

Fiber can slow the rate of carbohydrate absorption in the small intestine, which can lead to reduced glycemic and/or insulinemic indices [231,232]. Stachyose and raffinose are insoluble fibers that are present in defatted soy flour at about 1.4-4.1% and 0.1-1.2%,
respectively [74]. Both soy flour and soy milk powder contain about 2.5-3.0% soluble fiber but soy milk powder has considerably less insoluble fiber (14.2% vs. 21.3% for soy flour) [18]. Soluble fibers are more often associated with increasing the viscosity of the digesta, but stachyose and raffinose have also been shown specifically to slow the rate and extent of digestion [223]. Goñi and Valentín-Gamazo similarly observed a reduction in GI when they supplemented spaghetti with 25% chickpea flour, attributing their observation partly to an increase in indigestible compounds (including non-starch polysaccharides) from the chickpea flour [234]. This mechanism may in part contribute to the observed phenomena with soy addition to the soft pretzels.

Despite the attenuated glycemic response, the rate of insulin secretion did not significantly differ between ingestion of either pretzel. Although the carbohydrate concentration is diluted in the chyme, protein and lipid also stimulate glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoitropic polypeptide (GIP) which stimulate the release of insulin. This finding is consistent with the observation that high protein foods, such as lentils, elicit insulin responses greater that that predicted from the glycemic response [114]. Veldhorst et al. similarly showed that increasing soy protein concentration in a custard matrix increased insulinemia but not GI [9]. However, Pereira et al. showed that habitual fibrous diets can increase insulin sensitivity, in effect attenuating blood glucose levels after a meal [235]. In this study, because of the heterogeneity of the macronutrient composition in the pretzel matrix, we cannot correlate glycemia and insulinemia [236].

In the satiety study (Study 2), soy and wheat pretzels containing 239 kilocalories (1000 kJ) were consumed. Despite the soy pretzel having 5.2 more grams of protein and 2.2 more grams of fiber than the wheat pretzel, there was no statistical difference in the feeling of satiety (Figure 6.2). The large variation in satiety scores stems from the subjectivity of the assessment. To account for this, we used a cross-over design so that we could compare the
satiety values directly from each individual. A pattern was not observed between satiety declarations after consumption of the wheat or soy pretzel. There were likely counteracting factors that led to this observation. Protein both stimulates the release of cholecystokinin (CCK), which inhibits gastric emptying [237]. The increase in fiber can increase chyme viscosity, which leads to slowed gastric emptying and/or an increase in thirst which expands the stomach, leading to release of CCK, thus increasing satiety [238]. However, the wheat pretzels were larger in appearance due to the facilitated formation of air cells from higher gluten concentrations, and may have subconsciously increased satiety [239]. A study that includes a larger number of participants and can control for physical activity, food intake on days before the experiment, alcohol consumption, and sleep amount and quality may be able to detect subtle differences, if there are indeed any, between the satiety of these snack foods.

In order to control for the initial hunger level of the participants, the satiety study was designed so that the soy pretzel was consumed as the first meal of the day (breakfast) rather than as a snack. It has been estimated that university students, who comprised the majority of this participant pool, consume approximately 15-18% of their total energy intake from breakfast [240]. Assuming a 2000 kcal diet, the 249 kcal soy pretzel might have been less food than their normal breakfast, resulting in insatiety for both pretzel varieties. Hence, future studies investigating satiety of a soy snack food in between meals or as a more appropriately sized breakfast may avoid this complication.

Due to the water-binding abilities of soy protein [13] and the potential for the indigestible carbohydrates to increase chyme viscosity [238], we hypothesized that the soy pretzel would lead to a higher consumption of water that may contribute to increased satiety. However, the amount of water consumed was similar with the consumption of both pretzels.
6.5. Conclusion

The addition of 27.2% soy ingredients to a soft pretzel snack food can significantly decrease the GI without affecting consumer acceptability or satiety. These results show that it may be possible to supplement a variety of snack foods with soy at high enough quantities to achieve lower postprandial glycemia while maintaining favorable sensory characteristics.
Figure 6.1. Postprandial glycemia (a) and insulinemia (b) vs. time averaged across 12 participants (●=soy, ○=wheat). Error bars represent the standard deviation of participant distribution.
Figure 6.2. Satiety values for the participants as measured by AUC for satiety score vs. time relative to the baseline. Participants are ordered in descending order for wheat satiety values.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Source</th>
<th>Soy Pretzel (g/100 g)</th>
<th>Wheat Pretzel (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant Yeast</td>
<td>Lesaffre Yeast Corporation, Milwaukee WI</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Bread flour</td>
<td>ConAgra Mills, Omaha NE</td>
<td>40.97</td>
<td>56.40</td>
</tr>
<tr>
<td>Vital Wheat Gluten</td>
<td>Bob’s Red Mill, Milwaukie OR</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Dough Conditioner</td>
<td>The Prepared Pantry, Rigby ID</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Soy Flour</td>
<td>ADM, Protein Specialties Division, Decatur IL</td>
<td>11.53</td>
<td>-</td>
</tr>
<tr>
<td>Benesoy Soymilk Powder</td>
<td>Davansoy, Inc., Carroll IA</td>
<td>3.84</td>
<td>-</td>
</tr>
<tr>
<td>Iodized Salt</td>
<td>US Foodservice, Inc., Columbia MD</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>Pure Granulated Sugar</td>
<td>US Foodservice, Inc., Columbia MD</td>
<td>2.26</td>
<td>2.26</td>
</tr>
<tr>
<td>Vegetable Shortening (Crisco®)</td>
<td>The J.M. Smucker Co, Orrville OH</td>
<td>1.81</td>
<td>1.81</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>37.53 ± 0.90</td>
<td>37.49 ± 0.90</td>
</tr>
</tbody>
</table>

Table 6.1. Ingredients used to produce wheat and soy soft pretzels.
<table>
<thead>
<tr>
<th></th>
<th>Study 1</th>
<th></th>
<th>Study 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic/Insulinemic Index</td>
<td>Soy</td>
<td>Wheat</td>
<td>Soy</td>
<td>Wheat</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>332.1</td>
<td>270.8</td>
<td>239.0</td>
<td>239.0</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>5.2</td>
<td>3.4</td>
<td>3.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>54.7</td>
<td>51.4</td>
<td>39.4</td>
<td>45.4</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>4.7</td>
<td>1.4</td>
<td>3.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>17.2</td>
<td>8.1</td>
<td>12.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Total soy isoflavones (µmol)†</td>
<td>71.0</td>
<td>-</td>
<td>51.1</td>
<td>-</td>
</tr>
<tr>
<td>Total weight consumed (g)</td>
<td>72.9</td>
<td>62.0</td>
<td>52.4</td>
<td>54.5</td>
</tr>
</tbody>
</table>

*Table 6.2.* Nutritional composition of the pretzels for studies 1 and 2. †Isoflavone content was obtained from a previous study with the same soy ingredients [2].
Table 6.3. The numerical values for postprandial glycemia and insulinemia vs. time in Study 1, $n = 12$ participants. An asterisk (*) next to the soy glycemia value indicates statistical differences between soy and wheat values for a given time point. There were no significant differences between any of the insulinemia values for any given time point.
Chapter 7

The Type and Amount of Lipid in Soy Dough Modulates the Physiochemical Properties of a Soy Pretzel Roll

Amber L. Simmons & Yael Vodovotz

Abstract

Lipids provide essential functionality for the development of the crumb structure in bakery products. Herein, the effects of the type and amount of lipid on the water dynamics of soy dough and the physical properties of a baked soft pretzel roll were explored. Canola oil and ground almond at 2.9% and 6.0% added lipid were compared to shortening as a lipid source in soy soft pretzels. Soft pretzel crumb from all formulations exhibited about 40-43% moisture with a little more than half of the water present as “freezable” water. While desorption of water was observed at 23-40°C in addition to near 60°C for the shortening and ground almond samples, samples made with canola oil exhibited only water desorption at only the latter temperature. MRI imaging of the soy pretzel dough revealed greater $T_2$ relaxation times with increasing quantities of lipid, especially with canola oil, but to a much lesser extent with ground almond. Textural analysis of the crumb showed that an increase from 2.9% shortening to 6.0% shortening decreased the firmness and chewiness. Canola oil increased firmness and chewiness at 2.9% but reversed those effects at 6.0% compared to the 2.9% shortening formulation. Ground almond increased the firmness and chewiness with increasing concentrations of almond. The springiness and the cohesiveness of the crumb of all the pretzels were about $7.83 \pm 0.66$ mm and $0.64 \pm 0.06$, 

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respectively, regardless of lipid type and amount. These data suggest that there is a portion of the oil in the almond is not accessible to the dough or crumb matrix, thereby exerting different effects on the physical properties of the dough and crumb compared to addition of the oil as a pure source.

7.1. Introduction

Soy bread and soft pretzels are functional foods that are currently being evaluated for the treatment and prevention of disorders such as diabetes, cardiovascular disease, and cancer [28,241,242]. Macromolecular components of soy including high amounts of high quality protein and dietary fiber offer health benefits. Additionally, micronutrients including isoflavones, saponins, lignans, phytosterols, and others that could be additionally be contributing human health [243]. A soy soft pretzel offers a unique soy delivery system that has commercial potential at large scale venues where convenient preparation is preferred.

Lipids serve an essential structural role in the production of bakery products. They lubricate the gluten strands and adsorb to the gas-lipid interface of gas cells, allowing the bubbles to develop and be evenly distributed in the crumb [67]. Solid fats (traditionally lard, now more commonly shortening) promote a lighter, more homogenous crumb than liquid oils (review [68]). It is thought that oils do not adhere to the surface of gas cells as well as solid fats do, and therefore promote a less consistent distribution of gas cells and gluten in the dough. An unrefined lipid source, for example ground nuts, is unique in that it is composed of mostly unsaturated oils, but is present in a solid matrix. It is unknown if oil from ground nut can participate in molecular interactions that promote the growth of gas cells and serve the essential role of lipids in the developing crumb.

As a representative unrefined lipid source for use in these studies, ground almonds were chosen. Almonds contain approximately 50% fat by weight [88] so, in order to achieve the 2.9% traditional lipid amount, a soy bakery product requires 5.5% ground almond (w/w). Almonds are
a particularly appealing ingredient in the soy matrix because of their natural \( \beta \)-glucosidase activity on soy isoflavones. The soy soft pretzel used in these experiments contains about 40 mg of soy isoflavones per 59 g pretzel (see Chapters 6 and 8). Efficient uptake of isoflavones is necessary for them to deliver their health benefits, however, isoflavones in the aglycone form, rather than their native glucoside form, are taken up more efficiently into enterocytes in the small intestine \[20\]. A soy pretzel made with 5.5% ground, raw almonds produced a product with more than 25% aglycones, double the amount in soy bread (see Chapter 8). It was hypothesized that ground almonds could be substituted for shortening as the lipid component in the soy pretzel while increasing the amount of isoflavones present as aglycones and maintaining favorable crumb texture.

The lipids used in this study- shortening, canola oil, and ground almond- are all present as mainly as triglycerides, however, the chemistry of the various fatty acid side chains may interact differently with the matrix of the dough, leading to differences in gas cell formation, crumb structure, and/or crumb texture. Vegetable shortening is partially hydrogenated vegetable oil. It is solid at room temperature, and is often present in the \( \beta \) or \( \beta' \) crystal structure \[69\], meaning that it is more tightly packed in a tilted chain orientation, yet can vary between being highly ordered and having some disordered chains, depending on the dispersion of air bubbles and the shortening’s unique fatty acid structure. The optimal shortening in bread making has a melting point slightly higher than the proofing temperature (about 40°C), so it is present as a thin semi-solid during proofing. It is hypothesized that the shortening adsorbs to the gluten structure and to the surface of air bubbles, thereby strengthening the dough and retaining gas produced during proofing \[68\] (and references within). It is thought that the solid structure of the shortening is essential for its function, as the lipid crystals themselves have been observed at the surface between fat and water and fat and air \[67\]. Canola oil, in contrast, is liquid at room temperature and during proofing, thereby exhibiting no crystal structure. It does not adsorb to the macromolecular matrix or to the interface of gas cells as well as shortening, and thereby does not
promote gas retention as well shortening [67,68]. The translational and rotational mobility of the lipid molecules in the canola oil are hypothesized to be greater than in shortening, and therefore hydrophobic interactions may more strongly dictate their orientation. Lipid from almond is mostly unsaturated fat, and would be liquid oil if it were not bound in the lipid matrix. However, it is rotationally and translationally hindered, and it is unknown if it is even available for deposition gluten strands for lubrication or on the surface of gas cells during proofing.

There is some evidence that polar lipids (e.g. phospholipids) improve crumb texture due to interactions with gas cells. In fact, it was observed that the equivalent loaf volume can be achieved with 0.5% polar lipids from oat as with 3.0% shortening [70]. Soybean oil per se contains soy lecithin, a mixture of phospholipids that can improve the extensibility and elasticity of the dough which, in turn, improves loaf volume and crumb texture. It is unknown if the polar lipids within the added lipid source can aid in crumb development. Polar lipids have not been detected in vegetable shortening [71], and canola oil and almond oil contain a low amount of polar lipids (about 4.5% in canola oil [72] and about 3.7% in mature almonds [73]).

In order to assess the physiochemical effects of various lipid constituents on a soy soft pretzel and the pretzel dough, pretzel rolls were prepared with 2.9% or 6.0% lipid from shortening, canola oil, or ground almond. Pretzel rolls were formed instead of the traditional twisted shape in order to produce congruent samples of crumb for textural analysis. The crumb was subsequently assessed for moisture content, percent “freezable” water (FW), percent “unfreezable” water (UFW), and textural properties including firmness, springiness, chewiness, and cohesiveness. Unleavened pretzel dough was assessed via magnetic resonance (MRI) in order to probe the effects of the lipid types and amounts on the microscale interactions of the water within the dough matrix.

In order to assess moisture content of the crumb of a baked pretzel as well as the distribution of the water, thermogravimetric analysis (TGA) was utilized. The different types of levels of lipids were not expected to significantly change the total moisture content (almonds are
less than 5% water [88]), but canola oil was expected to affect water distribution. It has been observed that an increase in lipid leads to a decrease in total moisture requirements in dough, since the lipid itself binds to gluten protein and starch granules, reducing water adsorption by these molecules [244,245]. Therefore, it was hypothesized that canola oil compete with water within the matrix, partitioning the water into droplets that have less interaction with the gluten matrix and thereby allowing the water to evaporate at a lower temperature (see Chapter 4). Because lipid in almond is potentially trapped in the nut matrix, it was not expected to form large droplets. Therefore, lipid from ground almond was expected to not interfere with water distribution, though the additionally fiber was expected to bind excess water.

Differential scanning calorimetry (DSC) is a useful tool for the assessment of thermal transitions, including ice to water or melting of amylose-lipid complexes [246]. In this study, DSC was utilized to quantify the “freezable” water (FW) and “unfreezable” water (UFW) contents of the pretzel crumb. Very little research has been done on the effect of the added lipid on the FW and UFW distribution. Lodi et al. did not observe a difference in the proportion of FW and UFW in soy-almond bread compared to soy bread [33], so it was not expected for there to be differences between the shortening and almond pretzels.

In order to observe the textural effects of the lipid component on the pretzel crumb, textural properties were assessed with a double-compression test that simulates mastication. Due to facilitated gas formation and structural support of the gas cells [68], it was hypothesized that shortening would result in the softest crumb, with 6.0% shortening exhibiting the softest crumb of the 6 variables evaluated. It was hypothesized that canola oil would result in the hardest crumb due to a less developed gas cell system [68]. The crumb firmness of fresh soy bread has been shown to be decreased with the addition of almonds, presumably due to lipid functionality and higher loaf volume with added almond [33]. These results were hypothesized to be mimicked in these studies, and it was thought that the firmness of the crumb in the almond formulations would lie in between those of the shortening and canola oil formulations.
Lastly, in order to provide insight into the properties of the water in the dough that are revealed in the baked product, $T_2$ relaxation times in the dough were acquired with magnetic resonance imaging (MRI). Solid and liquid fats can be distinguished via relaxation properties [247] and can have important implications in the functionality of the dough and the properties of the baked product. For example, MRI has shown to be instrumental in illustrating the lipid distribution in crackers [248]. Proton density images of laminated crackers exposed distinct layers of dough and fat, predicting better mouth feel and shelf life compared to non-laminated crackers [248]. Lodi et al. did not observe large differences in the water states between soy bread and soy bread containing almond [14], but is unknown how the different lipid components will affect the water mobility in the dough and be able to predict physical attributes of the baked product.

The purpose of these studies was to assess the total content and distribution of water and the textural properties of the crumb of a soy soft pretzel made with a solid fat (vegetable shortening) compared to a liquid fat (canola oil) and a liquid fat in its unrefined state (ground almond). Additionally, MRI images and $T_2$ relaxation data were acquired in pretzel dough in order to probe water dynamics to better understand the physiochemical interactions between the components of the soy dough/bread matrix.

7.2. Materials and methods

7.2.1. Pretzel preparation

Soft pretzels with 50% soy ingredients (dry weight) were produced with the ingredients in Table 7.1. Formulations included 2.9% or 6.0% lipid from shortening, ground almond, or canola oil; none of the formulations included more than one of these lipid sources. The pretzel inherently contains about 1.9% lipid before added lipid (Chapter 8) so, total, these pretzels contain about 3.8% and 7.9% total lipid. To make the sponge, the activated yeast, 175 g wheat flour, the wheat gluten, and the dough conditioner were combined with 172 g total water in a
standing mixer on low with the beater attachment. The sponge was proofed at about 43°C at 100% humidity (CM2000 Combination Module, InterMetro Industries Corp, Wilkes-Barre, PA). The remaining ingredients were added and the dough was stirred with the dough hook attachment, first on low until ingredients were moistened, and then on medium-high. After about 5 min, the lipid source was added. The dough was then stirred until it sheeted (about 5 min more). Pretzels were then formed by rolling 71.0 g of dough into slightly oblong balls. Stickier formulations were dusted lightly with wheat flour to facilitate roll formation. Balls were submerged in 1.0% sodium hydroxide solution (60-65°C) for 60 sec and placed in a lightly greased mini loaf pan. Rolls were proofed for 25 additional minutes (43°C, 100% humidity) then baked at 149°C for 16 min in a convection oven (Jet air oven, JA14, Doyon, Linière, Québec, Canada). Rolls were cooled on wire racks, stored in plastic bags overnight, and analyzed the following day. At least 4 pretzel rolls were prepared and analyzed per batch. One batch was prepared for each variable.

7.2.2. Dough preparation for MRI

In order to best assess the differences between types and levels of lipids in regard to water properties in the dough, soy pretzel dough was prepared with 2.9% or 10.0% lipid from shortening, ground almond, or canola. Dough was prepared by combining 189.55 g wheat flour, 57.55 g soy flour, 19.2 g soy milk powder, 4.6 g wheat gluten, 0.6 g dough conditioner, 5.0 g salt, 20.0 g sugar, and 188.5 g water. (Yeast was omitted to avoid changes in the matrix during spectrum acquisition.) The dough was then divided into 6 × 75 g portions. Lipid was then added- 1.6 g for 2.9% shortening or canola oil, 5.1 g for 10.0% shortening or canola oil, 3.3 g for 2.9% ground almond, or 10.4 g for 10.0% ground almond. Lipid was incorporated by kneading by hand, and then dough was placed in 6 dram glass vials. Vials were capped and then sealed with parafilm to prevent moisture equilibration.
7.2.3. Textural profile analysis (TPA)

Pretzel rolls were sliced into 2 adjacent 2.5 cm cubes that included pretzel crumb free of crust (Electric carving knife, Toastmaster, St. Louis, MO). Each pretzel crumb cube was subjected to a double compression test to 40% compression at a crosshead speed of 100 mm/min (Instron Universal Texture Analyzer 5542, Instron, Norwood, MA). Firmness, springiness, chewiness, and cohesiveness, and adhesiveness of the pretzel crumb were calculated using Bluehill 2 software version 2.17 (Instron, Norwood, MA) [189]. Firmness was taken as the maximum force at the first compression. Cohesiveness was the ratio of the energy to maximum load during the second compression to that at the first compression. At least 5 pretzel cubes were analyzed per treatment.

7.2.4. Quantification of “freezable” and “unfreezable” water

Moisture content and water desorption patterns were assessed using TGA (Thermogravimetric Analyzer Q5000, TA Instruments, New Castle, DE). Samples of pretzel crumb from 15.0-20.0 mg were taken from the center of a pretzel roll and subjected to a linear heat ramp from room temperature to 200°C at 10°C/min. Under the assumption that weight loss resulted solely from water evaporation [44], the weight of the sample at 150°C was subtracted from the initial weight to yield moisture content. The derivative of the thermogravimetric analysis curve (DTG) was calculated by Advantage for the Q Series, version 2.8.0.394 (TA Instruments- Waters LLC, New Castle DE, 2001-2007).

The amount of FW was quantified in each dough sample using DSC. Between 10 and 15 mg of pretzel crumb was placed in a hermetically sealed stainless steel pan with O-ring (PerkinElmer, Waltham, MA). The temperature was lowered to -50°C, held isothermally for 2 min, and then increased linearly at 5°C/min to 150°C. The peak near 0°C was integrated to yield the change of enthalpy (\(\Delta H\)) associated with the phase transition from ice to water [49]. The latent heat of fusion of water (333 J/g) was used to quantify the amount of FW in the dough.
sample [18,188]. Subsequently, the amount of FW was subtracted from the amount of total water to yield UFW.

7.2.5. Proton intensity and $T_2$-weighed images

MRI scans were performed the same day the dough was prepared. The experiments were performed at ambient temperature on a 4.7T/40 cm magnet controlled by a Bruker Avance Console (Bruker Biospin, Billerica, MA). The instrument was equipped with a 260 mm inner diameter gradient coil and a 200 mm inner diameter proton volume radiofrequency coil. For intensity reference, a phantom sample was prepared by combining water and deuterated water at a proportion that resulted in a similar intensity as in an average dough sample. Both the dough sample and the phantom were placed inside the coil. Five slices were imaged for each sample. MRIcro software (version 1.40, Chris Rorden) was used to observe proton intensity images. ImageJ software (version 1.42, http://rsb.info.nih.gov/ij) was used to observe $T_2$-weighed images and produce histograms of $T_2$ relaxation times. The counts from the five slices were summed for analysis. The first of the 8 images acquired in the $T_2$ relaxation experiment was used the produce the proton density-weighted image. Experiments were repeated on a separate day with a new batch of dough to confirm reproducibility.

7.2.6. Statistics

For quantity of total moisture, FW, and UFW as well as texture properties, an analysis was performed to fit the model $Y = \text{LipidType} + \text{LipidAmount} + (\text{LipidType} \times \text{LipidAmount})$, where $Y$ = the parameter at hand; LipidType = shortening, almond, or canola oil; LipidAmount = 2.9% or 6.0%; and LipidType $\times$ LipidAmount is the interaction between the two variables. Analysis of variance (ANOVA) and least-square means for the (LipidType $\times$ LipidAmount) interaction were calculated using SAS 9.1 (SAS Institute, Cary, NC). Statistical significance was
deemed at $p < 0.05$. Average ± standard deviation is reported throughout the text. MRI proton intensity and $T_2$-weighed images were analyzed qualitatively.

7.3. Results and discussion

7.3.1. Moisture content, FW, and UFW

Soy dough requires a greater amount of water to hydrate its components than traditional wheat dough due to the hygroscopic properties of soy protein and the greater amount of fiber [28]. Therefore, soy will affect the distribution and binding properties of the water in the baked product and have important consequences for textural properties as well as shelf life [249]. Soy soft pretzels were produced with lipid from shortening, canola oil, and ground almond at two different levels and assessed for total moisture content, percent FW, and percent UFW (Figure 7.1). Because the same amount of water was added to all the soy pretzel formulations and almonds have only about 5% moisture [88], the observed similarity in total moisture content was expected. This observation also suggests that there are no differences in water migration, distribution between the crust and crumb, or evaporation from the crust during baking. Water desorption was observed at low temperatures for the shortening and almond samples, but not for the canola oil samples (Figure 7.2). The absence of a peak between 23°-40°C could be justified by one of two potential explanations: 1) there is water that is only loosely bound to the matrix that exhibits a vaporization temperature lower than room temperature and this water quickly evaporated during sample preparation, or 2) water does not exist in the fraction that is easily-removed in canola oil samples. The data collected here cannot discriminate between the two possibilities. Also, in the water desorption thermograms, there is a peak near 60°C that represents the majority of the water evaporation for all formulations (Figures 7.2 and 7.3). The temperature of this peak was similar for the shortening and almond samples, but was lower for the canola oil samples ($p < 0.05$ when comparing canola oil samples to other samples; 2.9% canola oil and 6.0% canola oil samples were not statistically significant; Figure 7.3). Together, these
phenomena suggests that canola oil is disrupting the interactions between water and the matrix, perhaps binding itself to macromolecular structures, and allowing water to more easily evaporate from the matrix. All lipids used in these formulations are liquid above about 50°C, so it is possible that the state of the lipid at room temperature dictates lipid-lipid interactions (such as the size and distribution of fat crystals) and interactions between the lipid and other macromolecules in the product. The lipid in the almond is likely bound in the nut matrix and most of it is perhaps not involved in macromolecular interactions with the other ingredients. In humans consuming almonds, it has been shown that more mastication of almonds led to a higher in vivo bioaccessibility and less lipid in the feces [250], so it is expected that the lipid bound in the almonds here is also inaccessible to its surrounding environment. In a thorough study by Lodi & Vodovotz that assessed water properties in soy and soy-almond bread, three populations of water were defined by deconvoluting the desorption pattern [33]. Despite the presence of shortening in both their samples, and therefore higher lipid content of the soy-almond bread, only small temperature differences were observed in the peak rates of water loss between soy and soy-almond bread at all temperatures [33], similar to this study. Therefore, it is suggested that canola oil displaces some of the water in the matrix, but the almond oil that is subject to diffusion is not at high enough concentrations to infiltrate the matrix. The differences between solid and liquid fats in regard to their ability to form lipid-air interactions in bubbles during and after baking has been thoroughly studied [67,68] however, the interaction between the lipid and the other components of the crumb such as water and protein (including gluten) has not been well characterized.

Only trivial differences were observed in the partitioning of water into FW and UFW compartments (Figure 7.1). This suggests that, if canola oil is indeed displacing some of the water within the macromolecular matrix, it is not displacing UFW to redistribute that water to FW. Matuda et al. investigated the effects of vegetable shortening and different dough conditioners on the effect of FW and UFW in French bread dough [45]. They similarly did not
observe a change in UFW between dough with and without shortening. There are not any studies to our knowledge investigating changes in FW and UFW in dough, bread, or other bakery products made with different types of lipid.

7.3.2. Proton intensity and $T_2$-weighted images of pretzel dough

MRI allows the spatial resolution of water content and water mobility in a sample [14,251]. Proton density-weighted images minimize the effects of $T_2$ relaxation and correlate with the water content of the dough at each voxel. Because the dough had similar amounts of added water, it was expected that these images would look similar, and that is what was observed (Figure 7.4). In all samples, the water appeared to be evenly distributed in the dough on a centimeter scale. However, the mottled appearance indicates that the water content of each voxel is slightly different than neighboring voxels. This mirrors what was seen previously in MRI images of non-yeasted wheat and soy dough [251]. The average intensity of random, approximately 0.5 cm $\times$ 0.5 cm sections were assessed for all samples and, as expected, there was no difference in intensity between the perimeter and the interior of the samples, suggesting little to no migration of water during short-term (several hours) storage at room temperature.

Lodi et al. observed a water-fat chemical shift artifact in the crust of soy-almond bread due to the lipid signal surpassing the water signal. However, the greater water content in the dough prevents this problem (the water peak was much more intense than the lipid peak in a 1D spectrum assessed by NMR, unpublished).

$T_2$-weighed images report the proton mobility, which is assumed to project water mobility when the signal from lipid is low. The distribution of $T_2$ relaxation times in the 2.9% lipid samples was similar for all types of lipid, centered near 16 msec (Figure 7.5). Greater lipid content led to a lengthening of $T_2$ time with all types of lipid, which implies an increase in water mobility. Samples with canola oil and shortening exhibited much greater shifts in average and standard deviation compared to the almond samples. This is likely due to the inaccessibility of
the almond lipid to the dough matrix environment, as was observed in TGA. One possible explanation for this observation is that the immiscibility between the water and lipid leads to larger droplets of water which behave more similarly to bulk water than water in a viscous solution. The range of $T_2$ values observed here were more broad than those in soy and soy-almond bread [33] indicating that water properties are much less homogenous. Because dough has a greater moisture content than bread, it was expected to exhibit a broad range of water molecules with various properties.

7.3.3. Texture analysis

This soy pretzel formulation contains 50% soy ingredients (dry weight; Table 7.1) and, consequently, less gluten, more protein, and more fiber (and more water for optimal workability [28]). The changes in the macromolecular composition and microscale ingredient interactions led to changes in texture when comparing bakery products made with or without soy ingredients including firmness and chewiness [43,252]. However, it was unknown how the lipid component may affect the textural properties of a soy-based bakery product.

In Figure 7.6, the firmness, springiness, chewiness, and cohesiveness of the pretzel rolls are illustrated relative to the properties of the 2.9% shortening product. The values for the 2.9% shortening product were: firmness: $9.37 \pm 1.63$ N, springiness: $7.64 \pm 0.26$ mm, chewiness: $57.8 \pm 9.6$ N, and cohesiveness: $0.62 \pm 0.03$ (ratio). Although it was expected that the canola oil products would be much harder and chewier than the shortening products due to their inability to promote gas cell development [68], less than a 25% increase in firmness was observed when ground almond or canola oil was used instead of shortening (Figure 7.6). An increase in lipid content caused a 40% decrease in firmness for both the shortening and, unexpectedly, the canola oil products. There is a hypothesis that that shortening melts during the proofing and baking products [253], thereby providing a constant source of lubrication for bubble formation and strengthening for air retention; perhaps the high amount of lipid in the 6.0% canola pretzel roll
provided ample lipid for coating growing gas cells during proofing while the soy ingredients aided in gas cell stabilization. The higher amount of almond did not soften the crumb, corroborating TGA and water mobility data suggesting that the lipid in the almond is bound within the nut matrix. Similarly, though the lipid is more accessible in a paste compared to ground almonds, an increase in firmness and chewiness was observed with addition of different kinds of nut pastes to wheat bread [254]. The springiness and cohesiveness of the crumb were similar between all formulations (Figure 7.6). Similarly, no practical changes were observed in springiness was observed when comparing wheat and soy bread [252], suggesting that this property is very resilient to changes in formulation. The cohesiveness values were similar to what has been reported previously in soy bread [252], and these value were greater than wheat bread (about 0.61 for soy, about 0.49 for wheat [252]). Similar to the trends observed with firmness, chewiness was increased slightly with ground almonds or canola oil in place of shortening at 2.9% (Figure 7.6). Moreover, at 6.0% added lipid, pretzels made with shortening and canola oil exhibited a decrease in chewiness, likely due to a more airy structure. In contrast, pretzels made with 6.0% lipid from ground almond were chewier than all other formulations at 89.2 ± 14.1 N. Collectively, these data suggest that 1) the addition of ground almond to a soft pretzel in an amount-dependent manner and canola oil addition at 2.9% led a greater force requirement to deform the product initially and, because deformations are equally reversible with all formulations, more total force would be required to chew the product; 2) addition of more than twice the canonical amount of shortening provides a softer, less chewy crumb; and, interestingly, 3) addition of greater amounts of canola oil, for example 6.0%, reversed the increase in firmness and chewiness seen at 2.9% canola oil.

The $T_2$ relaxation times did not appear to correlate with textural properties, though the dough used for MRI did not include yeast and therefore did not include air bubbles. Future studies with dough including yeast may help elucidate the role that lipids play in gas cell formation [255].
7.4. Conclusions

The substitution of 100% oil for shortening has historically led to a decrease in quality of bread crumb [68]. However, as a denser and chewier crumb is an asset for soft pretzels compared to bread. The pretzel matrix can easily accommodate a significant amount of soy [241] and this work has shown that pretzels can accommodate different types and levels of lipids, though a sensory evaluation would need to confirm this. The different types (shortening, canola oil, and ground almonds) and levels (2.9% and 6.0%) of added lipid did not affect the total moisture content or compartmentalization of FW and UFW. However, the water in the samples with higher lipid exhibited greater water mobility, as seen with MRI. The lipid in the ground almond appeared to be bound in the solid nut matrix, thereby not influencing the sorption of water by components of the crumb, affecting the water mobility, or affecting the firmness or chewiness of the crumb.

In order to improve this study, it would be interesting to compare the pretzel roll formulations with pretzel rolls without any added lipid. Additionally, it would be interesting to measure the volume of the baked product in order to more completely describe the ease of gas cell formation during proofing. Lastly, especially since physical property and sensory evaluation on pretzels is severely lacking, sensory evaluation would elucidate the consumer acceptability of the pretzel rolls to aid in optimization of a healthy, consumer-acceptable snack food.
Figure 7.1. The percent of FW (white) and UFW (dark gray) in the 6 soy pretzel roll formulations. “2.9” indicates 2.9% lipid from that lipid source; S = shortening, A = almond, and C = canola oil. There were no statistical differences in total moisture content or percent FW or UFW between the formulations ($p > 0.05$).
**Figure 7.2.** Example water desorption patterns from samples with 6.0% added lipid. Black is shortening, dark gray is ground almond, and light gray is canola oil.
Figure 7.3. The peak of the first derivative of the weight loss curve for water that evaporated near 60°C. (* $p < 0.05$). “2.9” denotes 2.9% added lipid, “S” is shortening, A is almond, and C is canola oil.
Figure 7.4. Proton intensity-weighted MRI images of dough with different types and levels of lipids. These images show one of five slices acquired for each sample. For each pair of images, the left circle is the phantom (H$_2$O/D$_2$O) and the right image is the dough sample.
Figure 7.5. Histogram showing $T_2$ relaxation times for soy dough. Blue lines represent shortening samples, orange/brown lines depict almond samples, and green lines represent canola oil samples. The lighter hue represents the sample with 2.9% added lipid while the darker hue represents the sample with 10.0% added lipid.
Figure 7.6. Textural properties of the pretzel rolls including firmness, springiness, chewiness, and cohesiveness. The actual values were normalized to the 2.9% shortening pretzel roll (the traditional formulation). Formulations with 2.9% added lipid are solid white or gray bars; formulations with 6.0% shortening have black polka dots. Shortening (SHO) samples are white; almond (ALM) samples are light gray; canola oil (CAN) samples are dark gray. Different letters above the firmness and chewiness groups indicate statistical significance ($p < 0.05$, ANOVA). *There were no practical differences between springiness and cohesiveness values so, for clarity, results of statistical analysis are not indicated on the graph.
<table>
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<th>Amount per 1 kg batch</th>
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<td></td>
<td></td>
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<td>40.75</td>
<td>106.59</td>
</tr>
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<td></td>
<td></td>
<td>2.9A</td>
<td>41.27</td>
<td>107.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0A</td>
<td>19.50</td>
<td>51.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.9C</td>
<td>51.50</td>
<td>134.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0C</td>
<td>40.75</td>
<td>106.59</td>
</tr>
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<td>10.00</td>
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Table 7.1. Pretzel roll ingredients. *Wheat flour was adjusted to compensate for adding mass of the lipid source in order to maintain equal soy content between batches. †Almonds were ground fresh in a blender to a size <1.70 mm (no. 12 sieve, W. S. Tyler, Mentor, OH). “2.9” indicates
2.9% lipid added from that source, “6.0” indicates 6.0% lipid added from that source, S = shortening, A = almond, and C = canola oil.
Chapter 8

Retention during Processing and *in vitro* Bioaccessibility of Isoflavones in a Soy Soft Pretzel

Amber L. Simmons, Chureeporn Chitchumroomchokchai, Yael Vodovotz, and Mark L. Failla

Abstract

The lipid fraction of a model soy snack food, a soft pretzel, was optimized for isoflavone uptake in a Caco-2 cell model. Pretzels were prepared with no added lipid (1.9% endogenous lipid), 2.9%, or 6.0% added lipid from shortening, canola oil, ground almond, or ground hazelnut. Isoflavones were stable during pretzel production, with about 15% conversion of malonylglucosides to simple glucosides, perhaps due to partial alkaline hydrolysis. Ground almond, due to endogenous β-glucosidase activity, facilitated partial conversion of simple glucosides to aglycones during proofing leading to a decrease in bioaccessibility of isoflavones to about 75% compared to 80-85% with other types of lipids. Amount of lipid in the pretzel did not affect bioaccessibility. Increasing the added amount of lipid from 2.9% to 6.0% and/or substituting canola oil for shortening did not affect uptake or metabolism, but the higher amount of shortening appeared to decrease the transepithelial flux of isoflavones by differentiated cultures of Caco-2 cells. These results suggest that the form of the isoflavone, but not the type or amount of lipid in a soy bakery product, may affect bioavailability of isoflavones.
8.1 Introduction

Soy foods have attracted much interest due to their proposed health promoting benefits such as alleviating hypercholesterolemia, reducing post-menopausal symptoms, and reducing the risk of some types of cancer [138]. While soy foods have been consumed as part of the typical diet in East Asian countries for centuries, soy is increasingly being incorporated into the American diet [7]. Incorporation of soy into products that Americans regularly consume, such as carbohydrate-based snack foods, represents one strategy to further increase soy consumption among this population.

Key components of soy that contribute to health benefits include the relatively high amounts of protein, fiber, and soy isoflavones. Soy isoflavones have a low binding affinity for the estrogen receptor and exert biological effects such as reducing high cholesterol, preventing breast or prostate cancer, and regulating glycemia [138]. The efficacy of soy isoflavones for health hinges on their absorption, transformation into bioactive metabolites, and distribution to target tissues, i.e. their bioavailability. Differences have been observed in the absorption and metabolism of soy isoflavones in response to the delivery matrix, genetics of the individual and their gut microflora, gender, and composition of one’s normal diet [149,256]. The form of isoflavones may be another factor affecting their bioavailability. Isoflavones glucosides are not present in circulation [257] and the rate of absorption of isoflavone aglycones occurs at a greater rate from some [20,258] but not all [155,259,260] foods containing aglycones rather than their glucosides.

An interesting observation is that intestinal uptake of isoflavonoid aglycones, especially genistein, may depend in part on their incorporation into mixed micelles during small intestine digestion [2]. Also, type and amount of dietary fat can affect the extent of micellarization during digestion and absorption of lipophilic dietary compounds such as carotenoids and vitamin E [162,261].
We considered several factors for optimizing the isoflavone profile of a soy snack food. First, a soy soft pretzel was selected as a model. Processing conditions for the production of the pretzels (e.g. heat and changes in pH) can hydrolyze ester and acetal linkages of isoflavones to generate simple glucosides and/or aglycones [152]. Also, preparation of soft pretzels involves submersion in a strong alkaline solution (lye) prior to the final proofing and baking stages, possibly inducing alkaline hydrolysis of isoflavone glucosides on the surface of the pretzel. Because raw almonds contain natural β-glucosidase activity that converts simple isoflavone glucosides to aglycones [262], we hypothesized that the incorporation of ground almonds into the pretzel formulation would increase the aglycone content above that of other soy-based bakery products. This, in turn, would be expected to impact the stability of the isoflavones during processing and digestion, as well as their absorption.

Because isoflavone aglycones require incorporation into mixed micelles for maximal intestinal uptake [2], the composition of the mixed micelle may affect the rate and/or extent of uptake, which has the potential to be manipulated with the pretzel formulation. Smaller micelles have been shown to promote more efficient uptake of compounds into enterocytes [163]. However, size of the micelle is primarily affected by the ratio of bile acids to phospholipids and churning of the chyme, with limited impact of composition of the meal [163]. On the other hand, the composition of the lipid components in the mixed micelles (e.g. the degree of saturation and chain length of fatty acids), affects the intestinal cell uptake of hydrophobic compounds including carotenoids [162,164,261] and cholesterol [165]. To our knowledge, the effects of the type, amount, and delivery matrix of lipid in a soy product formulation on the micellarization and uptake of soy isoflavones have not been investigated. Herein, we investigated the effects of shortening compared to canola oil, another common lipid source in the food industry, and ground almonds.

The coupled in vitro digestion/human Caco-2 cell model provides a useful method in these studies [175,181]. This model facilitates estimation of the stability during simulated
digestion of dietary compounds, their partitioning in the aqueous fraction of chyme, and their uptake across the brush border surface of enterocyte-like cells, i.e. bioaccessibility of dietary compounds of interest. Caco-2 cells have been shown to metabolize isoflavone aglycones to their glucuronidated and sulfated metabolites, as in humans [168].

The specific aims of the experiments with soy pretzels was 1) to assess the stability of isoflavones during pretzel production and in vitro digestion, and 2) to compare the effect of the type and amount of lipid in the formulation on the bioaccessibility, metabolism, and efflux of soy isoflavones from soy pretzels using the coupled in vitro digestion/Caco-2 cell model. We hypothesized that the presence of ground almonds as a source of β-glucosidase would increase the isoflavone aglycone content of the pretzel and perhaps increase the extent of isoflavone uptake and trans-epithelial transport by highly differentiated cultures of Caco-2 human intestinal cells.

8.2. Materials and methods

8.2.1. Supplies

Reagents, solvents, and materials for cell culture were purchased from Sigma Chemical Company (St. Louis, MO) and Fisher Scientific (Fair Lawn, NJ), unless noted otherwise. Isoflavone standards were purchased from LC Laboratories (Woburn, MA). Fetal bovine serum (FBS) and antibiotics were purchased from Gibco Life Technologies (Grand Island, NY).

8.2.2. Preparation of pretzels

Soy soft pretzels were prepared as previously reported [12,241]. Pretzels were produced with and without exogenous lipid. The endogenous lipid content of the pretzel was 1.9 ± 0.2% as determined gravimetrically using the Folch method [264]. In order to prepare the pretzels, a sponge was prepared with activated yeast, 43% of the total wheat flour, wheat gluten, the dough conditioner, and 45% of the total water were proofed at 43°C, 100% humidity (CM2000
Holding/Proofing Combination Module; InterMetro Industries Corp., Wilkes Barre, PA). After 2 h, the remainder of the water and other ingredients were added with exogenous lipid being added last.

No more than one exogenous lipid was added per formulation. Shortening was added at either 2.9% dry weight (the traditional formulation) or at 6.0% dry weight. Pretzels also were prepared to contain either 2.9 or 6.0% canola oil instead of shortening. Ground almond was added to deliver 2.9% additional lipid (5.9% ground almond, dry weight, assuming almonds are 49% lipid [88]) or 6.0% additional lipid (12.2% dry weight). Nuts were ground fresh with a kitchen blender to <1.70 mm (no. 12 sieve, W. S. Tyler, Mentor, OH) before addition to the formulation. As a control that provided lipid in a similar form as almond oil without β-glucosidase activity, canola oil was added at 2.9 and 6.0% dry weight. Hazelnut was used as a control matrix for almond because it contains similar amounts of total, saturated, and unsaturated fat as almond [88], but has very limited β-glucosidase activity. The hazelnuts were also ground to <1.70 mm and added to achieve 2.9% and 6.0% added lipid (4.7% and 9.8% ground hazelnut, by weight, respectively, since hazelnuts contain 61% lipid [88]). The amount of wheat flour was reduced to offset the addition of lipid and non-lipid components of the nuts in order to maintain a consistent amount of soy components among all formulations.

Dough (71.0 g) was rolled into 60 cm ropes and twisted into a pretzel shape. The pretzel was submerged in 1.0% sodium hydroxide solution, 60-65°C, for 60 sec and then placed on a lightly greased baking sheet (Pam 100% Canola cooking spray; ConAgra Foods, Omaha, NE). The pretzels were proofed for an additional 25 min (43°C, 100% humidity) and then baked at 149°C for 16 min (JA14 Jet-Air oven; Doyon, Linière, QC, Canada). Pretzels were cooled and stored in individual pint size plastic bags overnight.

β-Glucosidase activity of almonds was determined before and after baking as detailed previously [153]. One unit of enzyme activity (U) was defined as hydrolysis of 1 μmol p-nitrophenol (pNP) per min.
8.2.3. In vitro digestion

Pretzels were manually torn into pieces smaller than 1.0 g. Pieces totaling 25.0 g were combined with 166.7 g of “salt solution” (120 mM sodium chloride, 6 mM calcium chloride, 5 mM potassium chloride). After softening for 10-15 min, the mixture was homogenized at a rate of 6500 rpm/min for approximately 5 min until large pieces were no longer visible (Ika T25 Digital Ultra-Turrax Disperser with S 25N – 18 G Dispersing Element, Ika Works, Inc., Wilmington, NC). Homogenates were decanted into 50 mL polypropylene tubes and stored at -20°C.

The in vitro digestion procedure is based on the method previously described by Garrett et al. [175] and further modified by Thakkar et al. [177] to include an oral phase in addition to gastric and small intestine phases. The oral phase slightly differed from Thakkar et al. [177] in that the protocol used here used 1.8 U α-amylase per g pretzel. Two individual pretzels from each batch were subjected to simulated digestion in triplicate for a total of six independent digestions per batch, and at least two different batches were analyzed per pretzel formulation to yield a total of n = 12 digestions per formulation. Additionally, the pH for the small intestinal phase was adjusted to 6.0 ± 0.1 rather than 6.5 ± 0.1. The resulting suspension after completion of the small intestinal phase of digestion is referred to as “chyme” throughout the remainder of the text.

Duplicate aliquots (10 mL) of chyme were centrifuged at 5,000 × g for 45 min at 4°C (Beckman Coulter, Brea, CA) to separate soluble from undigested materials. The supernatant or aqueous fraction was collected for analysis and use in Caco-2 cell culture.

8.2.4. Cell culture

Differentiated monolayer cultures of Caco-2 human intestinal cells (HTB-37, American Type Culture Collection, Rockville, MD) were used to investigate the uptake, metabolism, and trans-epithelial flux of soy isoflavones. Details for the growth, maintenance, and experimental
use of these cultures are described elsewhere [22,175]. For experiments, cultures of Caco-2 cells were used between passages 26-34.

8.2.5. **Cellular uptake of isoflavones**

The aqueous fraction of the chyme was filtered (0.22 µm pores; Millex GP, PES, Millipore Ireland Ltd., Tullagreen, Cork, Ireland) before diluting with DMEM, pH 6.5, containing 500 µM phenol red to yield “test medium”. β-Glucosidase activity of gut microbes was mimicked by addition of 3 U/mL almond β-glucosidase for conversion of isoflavone glycosides to their respective aglycones.

Spent medium was aspirated from cultures and the monolayers were washed once with DMEM medium before addition of test medium to the apical compartment. The basolateral medium consisted of DMEM without phenol red and supplemented with 1.0% FBS and 1.0% non-essential amino acids. Cultures were incubated at 37°C in humidified atmosphere of 95% air:5% CO₂. Test medium was also incubated in wells without Caco-2 cells to assess the stability of the isoflavones. After 4 hr, apical and basolateral media was collected into 15 mL polypropylene tubes and centrifuged at 800 × g (GR412 Jouan, Winchester, VA). Supernatants were stored at -20°C until analysis. Monolayers were washed once with ice cold phosphate buffered saline (PBS), pH 7.4, containing 2 g/L albumin and then twice with ice cold PBS, pH 7.4. Cells were collected in cold PBS and centrifuged (800 × g, 4°C). The supernatant was discarded and the pellet was stored at -20°C until analysis.

Protein content of the cell pellet was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce BCA Protein Assay, Thermo Scientific, Rockford, IL). Barrier integrity of the monolayers grown on inserts was assessed by quantifying paracellular flux of phenol red from apical to basolateral medium. The flux of phenol red from the apical to basolateral compartments did not exceed 0.012 %/cm²/hr for monolayers used in these experiments.
8.2.6. Assessment of phase II metabolites in the spent media and cells

Thawed cell pellets were resuspended in 1.0 mL PBS, pH 7.4, and lysed by sonication (2 × 10 sec, amplitude setting 50; Vibra Cell, Sonics & Materials, Inc., Danbury, CT). In order to quantify isoflavones that were metabolized to glucuronidated and sulfated metabolites, aliquots of spent media and cell lysates were treated with either glucuronidase (β-glucuronidase, Type HP-2: from Helix pomatia) or sulfatase (Type H-1, from Helix pomatia). Both enzyme preparations exhibit glucuronidase, sulfatase, and glucosidase activity [257]. Due to co-elution of endogenous contaminants in the enzyme preparations, results using the “glucuronidase” preparation were used to estimate the phase II conjugation of daidzein and “sulfatase” was used to assess phase II conjugation of genistein. Cell sonicates or media were diluted with an equivalent volume of sodium acetate buffer, pH 5.5, with glucuronidase at a final concentration of 2.5 µL/mL, sulfatase at 5.42 mg/mL, or no added enzyme. Reaction tubes were incubated at 37°C in a shaking water bath (85 rpm) for 2-3 h for the sulfatase and no enzyme treatments or 16-20 h for treatment with glucuronidase. Total isoflavone content was calculated for the samples treated with either enzyme (isoflavones + phase II conjugates as aglycones) or buffer only (isoflavones that are not phase II conjugates). The difference between the two provided an estimate of phase II conjugates of isoflavones in samples.

8.2.7. Isoflavone extraction

Isoflavones were extracted from raw soy flour, soy milk powder, wheat flour, homogenized pretzel, chyme, aqueous fraction of the chyme, test media, stability samples, spent media, and cells according to Walsh et al. [2]. Extracts were dried under a stream of nitrogen gas and stored at -20°C until analysis.
8.2.8. Isoflavone quantification using high performance liquid chromatography (HPLC)

The dried isoflavone samples were resuspended in 80% methanol (400 µL), vortexed briefly (Vortex Genie 2, Fisher Scientific, Fair Lawn, NJ), and placed in a sonicating water bath for 60 sec (B12 Ultrasonic Cleaner, Branson Cleaning Equipment Co., Shelton, CN). The solutions were syringe-filtered (0.2 µm × 13 mm polyethersulfone filters, Fisher Scientific, Fair Lawn NJ) into HPLC vials with inserts (Verex Vial Kit, Phenomenex, Torrance, CA). Isoflavones were separated using a Symmetry C18 3.5 µm, 4.6 × 75 mm column with an inline Symmetry® C18 5µm, 3.9 × 20 mm guard column (Waters Corp., Milford, MA) held at 30°C. Isoflavones were quantified with a Waters 2695 Separations module with Waters 2996 Photodiode Array Detector (Waters Corp., Milford, MA). The samples were stored in an autosampler at 10°C. The injection volume was 10 µL for samples other than cells, for which 50 µL was injected. The mobile phase initially consisted of a mixture of acetonitrile (Solvent A) and 1% acetic acid in water (Solvent B) at a flow rate of 1.0 mL/min. The solvent gradient began at 10% A, 90% B with the gradient increasing linearly to 35% A from 1 to 23 min, to 75% A at 26 min, and returning to 10% A at 30 min to equilibrate for 5 min between samples. The PDA detector monitored a spectral range of 210-400 nm, and the chromatograms for isoflavone standards and samples were analyzed at 254 nm. Isoflavone standards for aglycones and simple glucosides were used for peak identification and quantification. Amounts in test samples were quantified by comparison of the area under the curve with 5 point standard curves for standards. At a signal to noise ratio of 5 to 1, the limit of detection was approximately 0.07 nmol/mL. Published retention times and spectra were used for identification of malonyl- and acetyl-glucosides [2] and standard curves for their corresponding simple glucosides were used to estimate the concentrations.
8.2.9. Data analysis

Statistical analyses was performed to fit the model $Y = \text{LipidType} + \text{LipidAmount} + (\text{LipidType} \times \text{LipidAmount})$, where $Y$ = the parameter of interest; LipidType = shortening, canola oil, almond, or hazelnut; LipidAmount = 0%, 2.9%, or 6.0%; and LipidType $\times$ LipidAmount is the interaction between the two variables. Analysis of variance (ANOVA) and least-square means for the (LipidType $\times$ LipidAmount) interaction were calculated using SAS 9.1 (SAS Institute, Cary, NC). Statistical significance was deemed at $p < 0.05$. Average ± standard deviation is reported throughout the text.

8.3. Results and discussion

8.3.1. Isoflavone retention and stability during production of soy soft pretzels

In order to assess the impact of standard pretzel processing on the profile and retention of isoflavones in a soy soft pretzel, the total amount of isoflavones and the isoflavone profile were first measured in the raw soy ingredients (Table 8.1 with representative chromatograms in Figure 8.1). Defatted soy flour and soy milk powder were combined in a ratio of 2:1 in the ingredient mixture [12]). In the soy ingredients, 51.1 ± 5.6% of the isoflavones were members of the genistein family, 44.9 ± 4.7% were members of the daidzein family, and 4.0 ± 0.6% were members of the glycitein family. This is in accord with other reports that show that the quantity of isoflavones in the genistein family is similar to or exceeds that in the daidzein family in soy foods [2,127,265]. By isoflavone form, the soy mixture contained approximately 57% malonylglucosides, 5% acetylglucosides, 31% simple glucosides, and 6% aglycones. This profile is similar to previous reports that the soy flour has 1-3% isoflavone aglycones [2,266] and soy milk powder has about 10% aglycones due to hydrolysis during processing [2,127]. Both ingredients used in these studies had a greater total amount of isoflavones than we reported previously [2], and this difference may be explained by variability between soybean crops and product batch [267,268].
After dipping in lye, proofing, and baking the raw ingredients to produce the pretzel, 93.2% isoflavones were recovered and the isoflavone profile was altered in a manner that is consistent with the preparation of soy bread [2]. More specifically, the percentage of malonyl- and acetyl-glucosides in a soy soft pretzel decreased to 38% and 4%, respectively, while the percentages of simple glucosides and aglycones increased to 41% and 17%, respectively. These changes in isoflavone profiles were consistent across both genistein and daidzein families.

To determine how much dipping in lye itself affected conversion of malonyl- and acetyl-glucosides to simple glucosides or aglycones, pretzels were proofed and baked without dipping, after dipping in water, or after dipping in lye (Table 8.2). All pretzels contained about 80-100 µmol (42 ± 4 mg) isoflavones per serving of pretzel (one serving = 59 g). Based on the observation that the proportion of simple glucosides increased at the expense of the malonyl- and acetylglucosides with no change in aglycones, it appears that ester bonds of isoflavones occurred on and near the surface were hydrolyzed during dipping in lye [152]. The dip did not alter the amounts of isoflavones in each isoflavone family. That is, all pretzels, regardless of the type of dip, contained approximately 52%, 44%, and 4% of the isoflavones in the genistein, daidzein, and glycitein families, respectively (data not shown).

The pretzels in this experiment contained 11-12% isoflavone aglycones, slightly fewer than other pretzels produced for experiments in this report (17-18%). This is likely because the pretzels were prepared using a different batch of soy flour, and because the quantity and profile of isoflavones can depend on growing conditions of the soybean crop [267].

8.3.2. The effect of the type and amount of lipid on the isoflavone profile in the soy soft pretzel

In order to assess whether the type and/or amount of lipid affects the isoflavone profile, pretzels were prepared with either no added lipid or 2.9% or 6.0% lipid added as shortening, canola oil, ground almond, or ground hazelnut. Endogenous lipid content of the pretzel was 1.9 ± 0.2%. Ground raw almonds were tested because they possess β-glucosidase activity that
preferentially cleaves simple isoflavone glucosides to aglycones [262], and because almonds increase the relative amount of soy isoflavone aglycones in a soy bread matrix during proofing and baking [21]. The other sources of lipid (shortening, canola oil, and hazelnut) were not expected to affect the isoflavone profile in the pretzel because they lack β-glucosidase activity.

Pretzels contained approximately 40 mg of isoflavones per serving (59 g), regardless of formulation. Pretzels produced without added lipid or with shortening, canola oil, or hazelnut contained 15-18% of total isoflavones as aglycones. With addition of almonds (2.9% lipid) to the pretzel, the relative amount of aglycones increased to 28.5 ± 4.0% (p < 0.001 compared to all formulations without almonds, ANOVA) and was further increased to 32.3 ± 2.9% in pretzels containing 6.0% lipid from almond (p < 0.001 when compared to all formulations without almond). These increases in aglycone quantity resulted from proportional decreases in simple glucosides with no changes in malonyl- and acetyl-glucosides. This confirms that simple isoflavone glucosides are the primary substrate for almond β-glucosidase [262]. This observation also confirmed that almond β-glucosidase was active during proofing and baking [21]. Activity in raw almonds was 1.0 U/mg almond. However, there was no detected activity in baked pretzels, suggesting that further enzymatic cleavage of isoflavone glucosides would be likely be minimal during product storage and/or digestion.

8.3.3. Digestive stability and bioaccessibility

At least 95% of the isoflavones in the pretzels were recovered after oral, gastric, and small intestinal digestions, indicating stability during digestion (see Figure 1 for representative chromatogram). Stability of isoflavones has also been observed in soy bread digested in vitro [2] and after digestion of tofu in rats [161]. The mean relative amount of isoflavone aglycones in the chyme generated during digestion of pretzels without almond increased from 16.8 ± 1.8% to 21.6 ± 1.8%. Similarly, for pretzels made with ground almond, the relative amount of aglycones increased from 28.5 ± 1.1% to 32.8 ± 1.4% during digestion for formulations containing 2.9%
lipid from almond ($p < 0.05$) and from $32.3 \pm 0.9\%$ to $36.4 \pm 0.8\%$ with $6.0\%$ lipid from almond ($p < 0.05$). This increase in aglycone content in the chyme is likely due to acid hydrolysis of simple isoflavone glucosides and ester bonds [152]. The similar degree of increase in aglycone content in pretzels with and without added almond during digestion (about 4-5%) further endogenous $\beta$-glucosidase activity is during digestion of the pretzels is limited.

Bioaccessibility of isoflavones, as defined by their partitioning in the filtered aqueous fraction, was $80-85\%$ for all digested pretzels other than those containing almond (Figure 8.2). By comparison, the bioaccessibility of isoflavones from pretzels prepared with almond was about $75\%$ ($p < 0.05$ for digested pretzels with the two levels of almonds compared to samples without almond). Regardless of the formulation, the relative amounts of aglycones partitioning into the aqueous fraction was only $40-60\%$ whereas partitioning of isoflavone malonyl-glucosides and simple glucosides exceeded $87\%$ for all formulations (data not shown). Because isoflavone aglycones are more lipophilic than glucosides, optimal bioaccessibility may require the presence of greater amounts of bile extract for greater incorporation into mixed micelles [2,269] Indeed, Walsh et al. [2] showed that addition of increased bile extract during the small intestinal phase of digestion increases the bioaccessibility of isoflavones from soy bread. When assessing bioaccessibility of isoflavone families, the efficiency of partitioning in the aqueous fraction was greater for the daidzein family than the genistein family $82.9 \pm 0.4\%$ vs. $75.0 \pm 0.1\%$ ($p < 0.001$). This difference is likely due to the greater hydrophilicity of daidzein than genistein [270].

Prior studies have shown that the type and amount of lipid consumed in the food/meal affect the composition of mixed micelles and therefore affect the bioaccessibility of some compounds including carotenoids, cholesterol, and cocoa polyphenols [162,261,271-273]. For example, chain length of fatty acids [162] and degree of saturation [166] have been reported to influence micellarization of some compounds during digestion. Goltz et al. [261] observed increased absorption of carotenoids from a vegetable salad with higher levels of lipids, and that unsaturated fat promoted carotenoid absorption to a greater extent than saturated fat. The amount
of lipid added to pretzel formulations did not affect apparent bioaccessibility of isoflavones in digested soy pretzels. Huo et al. [162] reported that only 0.5-1.0% of total triglyceride as either triolein or canola oil was required for optimal micellarization of the majority of carotenoids from a vegetable salad during simulated digestion. Because the octanol-water partitioning coefficient is much higher for carotenoids (e.g. 17.6 for β-carotene) than for isoflavones (3.0 for genistein and 2.5 for daidzein [270]), it is not surprising that the presence of additional lipid in the pretzel formulations or substitution of other lipids for shortening minimally affected partitioning of isoflavones in the aqueous fraction.

Although it has been established that the form of the isoflavone affects the pharmacokinetics of absorption [20,156,257,258], it remains controversial if ingestion of isoflavone aglycones instead of as glycosides differentially affects the extent of absorption. The decreased bioaccessibility of isoflavones after simulated digestion of pretzels containing almond may affect the rate of absorption, but it is unknown if such a difference will affect total absorption in vivo. Walsh et al. [2] reported that doubling the amount of bile extract during the small intestinal phase of in vitro digestion of soy bread increased micellarization of isoflavone aglycones during simulated digestion of soy bread from 10-15% to 15-25%. The concentration of bile salts in the lumen of the small intestine increases from 2-5 mmol/L during fasting to 6-15 mmol/L following a meal and is affected by both the amount and composition of food ingested [274].

8.3.4. Cellular uptake, metabolism, and efflux of isoflavones in the Caco-2 cell model

Isoflavone glycosides are transformed into aglycones by human lactase phlorizin hydrolase (LPH) that resides on the brush border membrane of enterocytes [275], and by gut microbiota residing predominately in the colon, as well as in the small intestine [276]. Caco-2 cells express very limited β-glucosidase activity [269,277,278], and intestinal bacteria/archaea were not present in the cell cultures. Therefore, β-glucosidase was added to the filtered aqueous
fraction (3 U/mL) before addition to cultures. This effectively converted simple isoflavone glucosides, but not malonyl- or acetyl-glucosides, to aglycones. In test media, aglycone content increased from 1.31 ± 0.24 nmol/mL to 6.74 ± 0.56 nmol/mL, representing 13.5% and 60.1% of total isoflavones present in the apical compartment, respectively.

To compare the effect of source and amount of lipid in the soy pretzel formulation on the uptake, metabolism, and trans-epithelial flux of soy isoflavones, pretzels were prepared with either shortening at 2.9% or 6.0% or canola oil at 2.9% or 6.0%. Pretzels were subjected to in vitro digestion, and the filtered aqueous fraction of chyme was used to prepare test media containing β-glucosidase (3 U/mL). Due to differences in soy flour batches used in these experiments, the amounts of aglycones in test media prepared with aqueous fraction of digested pretzels with shortening and canola oil were 15-18 nmol/mL and 28-31 nmol/mL, respectively. After incubation with cells for 4 hrs, approximately 85-89% of isoflavones were recovered in the apical compartment regardless of the type or amount of lipid in the pretzel formulation. Slightly less than 50% of the isoflavones present in the apical compartment were conjugated with glucuronide, sulfate, or both, indicating that that these isoflavones entered the cell, underwent phase II metabolism, and effluxed across the apical membrane. As expected [269], less than 1% of the isoflavones in the well were present within the cells (0.05-0.15 nmol/mg protein). Greater than 98% of isoflavones added to the medium were recovered after 4 h incubation in wells without and with Caco-2 cells.

The isoflavones that were transported into the basolateral compartment were considered “bioavailable”. Total isoflavones in the basolateral medium after 4 h incubation for all experiments ranged from 17% to 30% of that added to the apical compartment system were present in the basolateral compartment after incubation (1-3 nmol/mL). Of these, ≤30% were present as phase II conjugates. These data are consistent with the results of Walsh & Failla [22], who reported the presence of 26% of equol in the basolateral compartment of a Caco-2 system after a 4 hr incubation time. Overall, these data are also similar to those of Murota et al. [269].
and Steensma et al. [278] who reported that approximately 35% and 30-40% of isoflavone aglycones were transported into the basolateral compartment by Caco-2 cells, respectively. It is interesting that Chen et al. [168] reported preferential efflux of sulfated isoflavones into the apical compartment and glucuronidated isoflavones into the basolateral compartment, but our choice of enzymes precluded such distinction.

The lower limit of isoflavones that were taken up during the course of the experiment was estimated by the sum of the isoflavones in the basolateral and cellular compartments as well as the conjugated isoflavones in the apical compartments. About 50-65% of total isoflavones appear to have been taken up, which is similar to the total amount of aglycones present in the system and is somewhat similar to previous reports when genistein, daidzein, and equol were added to the apical compartment [22,269]. Passive diffusion has been suggested to be the main route of isoflavones into cells [155,257].

It did not appear that either type (shortening or canola oil) or amount (added at 2.9% or 6.0% to yield 4.8% and 7.9% total) of lipid strongly affected transepithelial flux of soy isoflavones. Phase 2 metabolites of isoflavones efflux across the apical and basolateral membranes of enterocytes into the intestinal lumen by multi-drug resistant related proteins [279]. However, it is currently unknown if isoflavones aglycones (and their metabolites) are transported across the basolateral membrane via specific transport proteins or incorporated within chylomicrons. Whereas aglycones are detected only in very low amounts in circulation [257], first pass conjugation of isoflavone aglycones in the liver is likely [172]. The type and amount of fat available to enterocytes affects chylomicron assembly [280]. Both shortening and canola oil are composed of mostly C16 and C18 fatty acids (Table 8.3) and differ primarily in their degree of saturation. Studies by van Greevenbroek et al. [280] have shown that the chylomicrons are smaller and denser when Caco-2 cells are incubated with palmitic acid compared to oleic acid. This may affect packaging and secretion of isoflavones into circulation. Further studies
investigating the effects of fatty acid chain length are needed for a more thorough evaluation of the type of lipid on isoflavone bioaccessibility and uptake.

Daidzein was transported across the Caco-2 monolayer more efficiently than genistein. Although daidzein and genistein each accounted for approximately 50% of the total isoflavone equivalents in the test media, about 60-80% of the isoflavones in the basolateral compartment was daidzein. Reported relative rates of uptake, metabolism, and excretion of daidzein and genistein are inconsistent. In contrast with our results, Chen et al. [168] observed a greater flux of genistein than daidzein in a Caco-2 cell model, but Cassidy et al. [258] observed similar pharmacokinetic rates of trans-epithelial flux for both these compounds. However, in accord with our results, Xu et al. [281] reported that daidzein absorption from soy milk by adult women was greater than that of genistein. Similarly, King & Bursill [167] observed that the rate of increase in blood concentrations of daidzein exceeded that of genistein when a soybean flour-based meal was administered to healthy men. Individual differences in expression of brush border enzymes (ex. LPH), differences in gut microflora, and effects of food matrix likely contribute to observed differences in isoflavone uptake by enterocytes and transfer into circulation. More research is required to better understand the basis for reported differences in the efficiency of absorption of isoflavones from foods.

In conclusion, the soft pretzel provides a distinct soy delivery system with a relatively high percentage of isoflavone aglycones due to the large surface area of the soft pretzel, alkaline hydrolysis of ester linkages of isoflavones located on the surface of the pretzel to yield simple glucosides during the lye dip, and β-glucosidase activity from added almonds to convert simple isoflavone glucosides to aglycones. The addition of almond to the soft pretzel promoted partial conversion of isoflavones to aglycones during proofing, but this in turn slightly decreased the bioaccessibility of total isoflavones. The bioavailability of isoflavones from soy soft pretzels in an in vitro Caco-2 model did not appear to be affected by the type or amount of lipid in the soy soft pretzels, be it shortening or canola oil at 2.9% or 6.0% added lipid. Elucidation of the
relationship between the gut microbiota and isoflavones as well as details regarding the biology and biochemistry of isoflavone uptake and metabolism will help further optimize soy foods for clinical and general health benefits.
Figure 8.1. Representative HPLC chromatograms of isoflavone profiles in extracts from wheat flour, soy flour, soy milk powder, soy soft pretzel prepared with 2.9% shortening, chyme of a pretzel digested in vitro, and the aqueous fraction of the chyme for the same pretzel formulation. Isoflavones were identified using retention times and absorption spectra of standards from simple glucosides and aglycones and from published literature [2]. Isoflavones eluted in the following order: 1) daidzin, 2) glycitin, 3) genistin, 4) malonyldaidzin, 5) malonylglycitin, 6) acetyldaidzin, 7) malonylgenistin, 8) daidzein, 9) glycitein, 10) acetylgenistein, and 11) genistein. Acetylglycitin was not detected.
Figure 8.2. Addition of almond to pretzel formulation reduced the relative bioaccessibility of isoflavones. Data are mean ± standard deviation for at least 12 independent digestions of pretzels with indicated type and amount of lipid. NAL: no added lipid; SHO: shortening; ALM: lipid from ground almond; CAN: lipid from canola oil; HAZ: lipid from ground hazelnut. Amount of lipid from these sources added to formulations was either 2.9% or 6.0% lipid dry weight. (Endogenous lipid content from soy ingredients was 1.9 ± 0.2%). Presence of different letters above error bars indicates that differences in means are statistically significant (p < 0.001).
<table>
<thead>
<tr>
<th>Isoflavonoid</th>
<th>Soy Flour (nmol/g)</th>
<th>Soy Milk Powder (nmol/g)</th>
<th>Expected IFNs in Soy Pretzel</th>
<th>IFNs measured in Soy Pretzel</th>
<th>Individual IFNs (%)</th>
<th>IFN Families (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzin</td>
<td>896.7 ± 32.5</td>
<td>1734.8 ± 85.6</td>
<td>292.0 ± 12.1</td>
<td>350.6 ± 15.8</td>
<td>120.1</td>
<td>89.7</td>
</tr>
<tr>
<td>Malonyldaidzin</td>
<td>1977.4 ± 84.5</td>
<td>1795.3 ± 104.8</td>
<td>511.4 ± 23.7</td>
<td>299.8 ± 17.1</td>
<td>58.6</td>
<td></td>
</tr>
<tr>
<td>Acetyldaidzin</td>
<td>138.0 ± 18.3</td>
<td>115.7 ± 17.2</td>
<td>35.1 ± 4.8</td>
<td>29.4 ± 2.1</td>
<td>83.9</td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>148.0 ± 24.2</td>
<td>168.7 ± 30.6</td>
<td>40.5 ± 6.8</td>
<td>108.4 ± 3.4</td>
<td>267.6</td>
<td></td>
</tr>
<tr>
<td>Genistin</td>
<td>764.4 ± 37.8</td>
<td>1761.1 ± 79.2</td>
<td>267.3 ± 12.7</td>
<td>357.8 ± 17.4</td>
<td>133.8</td>
<td>101.3</td>
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<td>Malonylgenistin</td>
<td>2059.7 ± 77.9</td>
<td>2004.9 ± 96.8</td>
<td>541.5 ± 21.8</td>
<td>349.7 ± 18.4</td>
<td>64.6</td>
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</tr>
<tr>
<td>Acetylgenistin</td>
<td>209.9 ± 7.7</td>
<td>110.4 ± 10.9</td>
<td>49.0 ± 2.2</td>
<td>35.2 ± 2.7</td>
<td>71.7</td>
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</tr>
<tr>
<td>Genistein</td>
<td>112.6 ± 12.8</td>
<td>263.3 ± 20.7</td>
<td>39.6 ± 3.9</td>
<td>166.7 ± 6.7</td>
<td>420.7</td>
<td></td>
</tr>
<tr>
<td>Glycitin</td>
<td>154.8 ± 5.7</td>
<td>67.6 ± 34.5</td>
<td>35.3 ± 3.4</td>
<td>32.7 ± 3.5</td>
<td>92.8</td>
<td>65.3</td>
</tr>
<tr>
<td>Malonylglycitin</td>
<td>243.7 ± 25.0</td>
<td>89.2 ± 3.1</td>
<td>54.4 ± 5.2</td>
<td>28.7 ± 2.8</td>
<td>52.8</td>
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<tr>
<td>Acetylglucitin</td>
<td>80.2 ± 14.8</td>
<td>53.1 ± 11.5</td>
<td>19.5 ± 3.7</td>
<td>nd(^b)</td>
<td>0.0</td>
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</tr>
<tr>
<td>Glycitein</td>
<td>198.8 ± 10.8</td>
<td>35.9 ± 12.5</td>
<td>42.0 ± 3.0</td>
<td>37.3 ± 3.9</td>
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<tr>
<td>Total</td>
<td>6984.1</td>
<td>8200.1</td>
<td>1927.5</td>
<td>1796.3</td>
<td>93.2</td>
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</tr>
</tbody>
</table>

Table 8.1. Isoflavone content of individual soy ingredients and baked soy pretzel without almonds. \(^a\)Soy flour and soy milk comprise 18.5% and 6.2%, respectively, of pretzel dry weight. \(^b\)Not detected.
Table 8.2. Dipping in lye alters the isoflavone profile of a soy pretzel. Data are mean ± standard deviation for \( n = 6 \). Means in a row with different letters as superscripts are significantly different \((p < 0.05)\).

<table>
<thead>
<tr>
<th>Percentage of total</th>
<th>No Dip</th>
<th>Water dip</th>
<th>Lye Dip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malonylglucosides</td>
<td>47.5 ± 2.9%</td>
<td>47.0 ± 2.0%</td>
<td>40.3 ± 1.7%</td>
</tr>
<tr>
<td>Acetylglucosides</td>
<td>8.2 ± 1.1%</td>
<td>10.5 ± 0.8%</td>
<td>7.1 ± 0.8%</td>
</tr>
<tr>
<td>β-glucosides</td>
<td>32.2 ± 2.7%</td>
<td>30.3 ± 2.0%</td>
<td>41.4 ± 1.8%</td>
</tr>
<tr>
<td>Aglycones</td>
<td>12.0 ± 1.7%</td>
<td>12.3 ± 1.5%</td>
<td>11.2 ± 1.4%</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>Saturated fats</td>
<td>Mono-unsat.</td>
<td>Polyunsaturated fats</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
<td>-------------</td>
<td>---------------------</td>
</tr>
<tr>
<td></td>
<td>C14:0</td>
<td>C16:0</td>
<td>C18:0</td>
</tr>
<tr>
<td>Canola Oil</td>
<td>-</td>
<td>5.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Shortening</td>
<td>0.1</td>
<td>11.6</td>
<td>10.7</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>-</td>
<td>5.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Almond</td>
<td>-</td>
<td>6.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>0.5</td>
<td>10.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Table 8.3. The fatty acid composition of the lipid sources used in this experiment, as percent total lipid [88].
Chapter 9

Conclusion

The central hypothesis was that soy could benefit both the nutritional and functional quality of snack foods, using the soy soft pretzel as a model. In a narrower focus, it was hypothesized that the lipid component of the soy pretzel formulation could be manipulated to maximize the nutritional properties of the pretzel while maintaining high quality. Using a variety of approaches encompassing physics, material science, food science, in vitro nutrition, and in vivo human nutrition studies, it was shown that our hypotheses were correct in many arenas. Addition of soy to bread dough at 49% slightly increased the frozen shelf life of dough, leading to fewer changes in the dough and baked bread with increased frozen storage time (Chapter 4). The changes in the physical properties of the dough and bread were explained by showing that microscale migration of water from a “bound” to “free” state occurred slower in soy dough than in wheat dough (Chapter 5). In comparison to the wheat pretzel, the soy soft pretzel was shown to be a consumer-acceptable alternative with equivalent acceptability ratings (Chapter 6). Additionally, the snack was shown to accommodate different types and levels of lipid including shortening, canola oil, and ground almond (Chapter 7). β-Glucosidase activity in raw almonds efficiently converted a portion of soy isoflavones into aglycones during proofing, yielding a 2-fold increase in the proportion of aglycones (Chapter 8). Meanwhile, the soft pretzel maintained moisture content and distribution, springiness, and cohesiveness, and only exhibited only a slight increase in hardness and chewiness (Chapter 7). The fact that the lipid in almond was present in the liquid form rather than (ideal) solid form did not have a large effect on the physical properties
of the pretzels, possibly because a large portion of the lipid is inaccessible to the dough matrix. Soy isoflavones exhibited >90% recovery in the baked soft pretzel compared to the ingredients, denoting excellent stability through the preparation and baking process (Chapter 8). Soy isoflavones also demonstrated excellent stability through a simulated digestion protocol (Chapter 8). Only approximately 75% of the soy isoflavones from pretzels that were made with almonds were bioaccessible compared to about 82% in pretzels produced with other types of lipids (Chapter 8), but it is hypothesized that this percentage can be increased by consuming the soy food with lipid in the meal to increase bile secretion production and promote micellarization of the hydrophobic aglycones [2]. Conversion of the isoflavones from glucosides to aglycones promoted the uptake of isoflavones in an in vitro cell model, but the type nor amount of fat affected isoflavone uptake, metabolism, or transepithelial flux into the basolateral compartment (Chapter 8). In human studies, the soy soft pretzel exhibited a low glycemic index (39.1 ± 20.4) compared to the wheat pretzel (66.4 ± 15.3; Chapter 6), suggesting it to be a healthy option for people with diabetes [1] and for healthy people in preventing hyperglycemia. The soy soft pretzel offered a similar satiety value as the wheat pretzel when served as breakfast (Chapter 6) but, due to its relatively small size for a meal, the soy pretzel is hypothesized to be more satiating as a mid-day snack than the wheat-based pretzel. In summary, soy has shown aid in preserving the quality of bread dough during frozen storage as well as optimizing the nutritional quality of a soft pretzel.
Chapter 10

Future Directions

As the prevalence of obesity, diabetes, metabolic syndrome, and other nutrition-related disorders continues to escalate, is it crucial that nutrition scientists work together to identify the causes of these conditions, elucidate the mechanisms of etiology, and develop effective treatment and prevention plans for the future. Soy was shown to be an efficacious ingredient for the reduction of the glycemic index in a model snack food; this observation paves the way for follow up studies in order to increase the breadth of the impact of functional snack foods. Additionally, these studies evaluated a novel approach to maintain quality during frozen storage and elucidated the effects that lipid in the nut form can have on the physical properties of a dough matrix. Outlined here are several short-term and long-term research studies that build upon the knowledge gained.

10.1. Scaling up soy pretzel production

Pretzels were chosen as a model snack food because they are popular and reach a large amount and large variety of people. Because they are low in fat, pretzels are already considered a “healthy” option at large theme parks, sporting events, fairs, and other venues that require large-scale, convenient preparation. However, aside from a little bit of protein and a few B-vitamins [88], pretzels do not offer high quality nutrition. It would be interesting to introduce the soy soft pretzel to the public, perhaps at an Ohio State University Buckeyes football or basketball game, in order to assess the feasibility and the acceptability by the public. It would be important to
collaborate with marketers on this introduction in order to maximize sales and have the biggest impact. For example a “Soy Pretzel” may not sound appealing to many Americans, but “High Protein Pretzel” might, or perhaps it would be best to not advertise the change in vendor.

10.2. Pretzels for diabetics, pre-diabetics, children

We showed that the soy soft pretzel promoted an attenuated post-prandial rise in glycemia compared to the wheat soft pretzel in healthy adults. Consuming foods with a low glycemic index is strongly advised for people with diabetes, people with pre-diabetes, and people with a higher susceptibility to diabetes, as well as for healthy individuals [1]. People with diabetes have different physiology than people without diabetes; for example, they tend to exhibit greater post-prandial glycemia in response to a meal due to an impaired insulin response. It would be prudent to test the difference in post-prandial glycemia between the soy and wheat soft pretzels in people with diabetes in order to confirm the efficacy and promote the soy-fortified snack food as part of their diet.

Because snack foods are comprising as larger part of children’s diets [224], it would be interesting to evaluate the effects of soy addition on the glycemic index of the soft pretzel or any model snack food in children. Snack foods with soy could potentially be more nutritious substitutes for crackers, soft and hard pretzels, cookies, and others.

10.3. Incorporating soy into other bakery products

Because the soft pretzel served as a model snack food, an obvious future direction is to expand the line of soy-enriched products. In addition to the soft pretzel, soy bread [242] and a low moisture soy flat bread was developed [43], verifying that soy is useful in a large variety of bakery products. Popular foods that are currently made with white wheat flour and have great potential for feasibility include: hard pretzels (small and large), crackers, cookies, pasta, pizza
crust, brownies, cakes, tortillas, pita bread, bagels, doughnuts and other pastries, pancakes, muffins, bread crumbs used for coating, and breakfast cereals.

10.4. Insulinemic index

The soy soft pretzel led to an attenuated post-prandial rise in glycemia compared to the wheat soft pretzel with no difference in the insulinemic index. Because insulin is the chief hormone recognized to promote glucose uptake than thereby reduce blood glucose concentrations, it appears that insulin sensitivity was greater after the soy pretzel than after the wheat pretzel. It is known that protein stimulates insulin secretion [101], but the complex relationship between various hormones is not yet understood; it is unknown if the simple addition of protein to the pretzel can account for the difference in glycemic index. Many studies assess glucose tolerance/insulin sensitivity as a health parameter that can change over the course of months [282], but the effect of meal composition (especially macronutrients, at least initially) on the post-prandial “secretome” as well as hormone interactions has not thoroughly been assessed. It would be interesting to examine blood concentrations of insulin, glucagon, GLP-1, gastrin, cholecystokinin, neuropeptide Y, and other hormones to tease out the mechanism by which soy has the ability to attenuate post-prandial blood glucose.

10.5. Satiety experiments

Due to the greater amount of protein and dietary fiber, the soy soft pretzel was hypothesized to be more satiating than the wheat soft pretzel. However, the soy soft pretzel and the wheat soft pretzel were perceived to be equally satiating when consumed as the first meal of the day. In this study, in order to control for hunger, participants were asked to consume the pretzel as a breakfast after an overnight fast. The pretzels were likely not as large as typical American breakfasts (only 239 kcal), hence it is hypothesized that a larger pretzel, consuming
this pretzel as a snack instead of a whole meal, or consuming the pretzel as part of a meal may lead to a greater feeling of satiety with soy compared to without soy.

10.6. The effects that the food matrix may play on the health benefits of soy

Soy is an extremely versatile food, existing in edamame, roasted nuts, milk, flour, tofu, yogurt, refined protein nuggets, and many other forms. Depending on the food matrix, the macro- and micronutrients of soy could be processed by the body to a different extent or at a different rate, thereby exhibiting different physiological responses and promoting different health outcomes. Aedin Cassidy’s laboratory in the U.K. have assessed the isoflavone concentrations in various soy foods as well as the absorbance [258]. However, it would also be interesting to look at the effects on the food matrix on mechanics of digestion, the hormonal response, the absorption of the macromolecules such as protein, and the absorption of other micronutrients in soy such as saponins.

On a broader scale, scientists have not closely looked at the “proteinemic index” (i.e. post-prandial protein concentrations in the blood). Is it beneficial to initiate a slow, steady intake of peptides and amino acids as opposed to a rapid intake?

10.7. $^{17}O$ NMR experiment on bread dough

Trends of changes in water mobility as calculated from $T_2$ relaxation measurements are ambiguous because the plot of $T_2$ vs. correlation time (the inverse of water mobility) traces a parabolic shape (Figure 3.5). When performing NMR experiments on bread dough, because water is such a small molecule, it is assumed that the correlation time of the protons in the water is smaller than the Larmor frequency and, therefore, an increase in $T_2$ time implies an increase in water mobility. This assumption is reliable in liquids, but is less dependable in viscous solutions [201]. In bread and bread dough, there exists very little evidence of this assumption being correct. I have proposed that the water in low moisture products have a correlation time that is greater.
than the Larmor frequency [15], but data for higher moisture produces such as dough remain ambivalent. In addition, to make proton relaxation behavior more complex, cross relaxation and exchange processes are strong [201,202]. A straightforward experiment that could help shed light on this research question is an $^{17}$O relaxation experiment in which dough or bread with a range of total moisture contents are assessed for $T_2$ relaxation times. Increasing moisture content will lead to an increase in water mobility and will therefore result in increased or decreased $T_2$ times, depending on the relationship between the correlation time of the water and the Larmor frequency. Oxygen in water does not exhibit cross relaxation or oxygen exchange, thereby making relaxation experiments easier to interpret [201]. Due to the low abundance of $^{17}$O, bread and dough would need to be enriched. This can be accomplished by either adding $^{17}$O-enriched water to the dough at 1-4% (which may be prohibitively expensive), or by incubating the sample with saturated salt solutions enriched with the desired enrichment of $^{17}$O [283]. Bread may need more enrichment than dough due to its lower total moisture content.

10.8. Introduction of gut bacteria into the in vitro digestion model

Soy isoflavones are extensively metabolized by gut bacteria, and individual differences in the gut microflora lead to vast differences in the identity of metabolites [284], rate of metabolism [20], and bioavailability [156]. Only recently is the gut microflora being explored to assess the potential differences on the potential health benefits of soy isoflavones [131,285]. About 35% of the Western population harbors bacteria the metabolize daidzein to equol, a compound that binds to the estrogen receptor with greater affinity than all other isoflavones, whereas a larger percentage of vegetarians and Asians produce equol [134] (and references within). Pure β-glucosidase (from almonds) was added to the test media to mimic bacterial activity, but hydrolysis was limited and metabolites such as equol were not formed (Chapter 8). Because of the crucial role that gut bacteria play in the metabolism of soy isoflavones, bacterial addition to the in vitro digestion model that was used would provide a much more accurate representation of
soy isoflavone metabolism. Based on my reading, I would recommend a static digestion model that would include the same oral, gastric, and small intestinal phases used herein, and then be followed up with inoculation of the sample with fecal material to simulate a colon phase [286] (review).

10.9. *Is there an advantage to consuming malonyl- and acetyl-glucosides?*

Isoflavones that are in the aglycone form are taken up at a faster rate and to a larger extent that isoflavone in the glucoside state [20]. On the brush border membrane of human enterocytes [275], and to a much lesser extent in Caco-2 cells [287], lactase phlorizin hydrolase (LPH) deglycosylates simple isoflavone glucosides into aglycones. The affinity of LPH for malonyl- and acetyl-isoflavone is thought to be low [288], so it is projected that isoflavones in these forms are “protected” from uptake in the small intestine and travel to the colon where bacterial metabolism and subsequent uptake occurs. Consequently, isoflavones delivered as aglycones are likely seldom converted to the more biologically active compounds (like bacteria-derived equol), potentially escaping biological action [131]. The benefits and dangers of equol are still under debate [131], and only future studies will help shed light on the optimal isoflavone *types and quantities* that individuals ought to be consuming.

10.10. *Purification of the H. pomatia preparation*

The *H. pomatia* enzyme preparations contained contaminants that co-eluted with daidzein or genistein in the HPLC chromatogram. It would be a nice undergraduate project to optimize a purification protocol to remove small molecule contaminants using a size exclusion column. This would simplify all future analyses of phase II metabolites of soy isoflavones.
10.11. Human study based on in vitro/cell work

There did not appear to be an effect of the level of lipid in the soy pretzel formulation on the uptake, metabolism, or efflux of soy isoflavones. Although the model was carefully designed to mimic the human digestive tract, the human digestive environment is more complex and possesses different types and amounts of enzymes than were used. (It also exhibits different inter- and intra-individual variability depending on many host factors). In addition, the phenotype and physiology of the enterocyte not identical to the Caco-2 cells. These cells differ in origin (Caco-2 cells are derived from the colon, not the small intestine) and utilize a different pathway for triglyceride biosynthesis [181] (and references within). Lastly, the complex relationship between gut bacteria and the host was not identically simulated, and therefore differences in soy isoflavone uptake and metabolism are likely to be different [284]. The coupled in vitro digestion/Caco-2 cell model is undoubtedly a valuable model for preliminary studies (including screening of compounds) and elucidating mechanisms since the system is easily controlled. However, only human studies will be able to ultimately resolve differences in the type and amount of fat on the uptake, metabolism, and efflux of soy isoflavones.

10.12. Sensory on the different types and levels of lipids

Within Chapters 7 and 8 there were 9 soy pretzel formulations that were evaluated for physical properties and bioaccessibility of isoflavones. It is important to evaluate the consumer acceptability of these products in order to predict their success in the market.

10.13. The use of non-traditional solid fats in baking to better describe the mechanism of lipid functionality

Shortening consists of mostly saturated fat, an “unhealthy” fat, and there is a drive to replace shortening with more heart-healthy oils [289]. Most of the literature on the functionality of lipids in baking highlights the benefits of shortening and lard over liquid oil due to their
relatively high melting point (both melt near 40°C) and solid structure at room temperature, thereby allowing lubrication of gluten, stabilization of gas cells, and other favorable functions during the proofing and baking process of bakery products. However, there are large differences in the fatty acid composition between shortening/lard and oils that have not yet been fully evaluated in their function during baking. For example, shortening and lard consist of about 25-40% saturated fat, whereas liquid oils consist of much less (canola oil and almond oil are 8% saturated fat) [88]. I propose to test the hypothesis that selected fats with a melting temperature between 25°C and 50°C will function optimally in a bakery product, leading to primary outcomes such as homogenous gas cell formation and high loaf volume. To support this hypothesis, I propose to evaluate the physical properties of bakery products made with coconut oil (86% saturated fat, $T_m = 25$°C), palm oil (71% saturated fat; $T_m = 35$°C), or cocoa butter (60% saturated fat; $T_m = 32$°C), with shortening as a control. Coconut oil and palm oil are considered healthier fats than shortening. They are composed mainly of medium chain fatty acids which, upon absorption, are transported via portal circulation to the liver rather than within chylomicrons in the lymph [290]. They are consequently more readily oxidized and less prone to be stored, which has been proposed as a dietary method to prevent obesity [290]. Cocoa butter is not considered healthy due to its ability to cause oxidative stress [291], but is a useful, readily available lipid that can be used to thoroughly evaluate this hypothesis.
Appendix A: The Ohio State University Institutional Review Board Application for
Acceptability and Preliminary Satiety Studies
**INITIAL REVIEW OF HUMAN SUBJECTS RESEARCH**

The Ohio State University Institutional Review Boards

Office of Responsible Research Practices (ORRP)
300 Research Foundation Building, 1960 Kenny Road, Columbus, OH 43210
Phone: (614) 688-8457  Fax: (614) 688-0366  www.orrp.osu.edu

<table>
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<tr>
<th>OFFICE DB</th>
<th>DATE RECEIVED:</th>
<th>DATE VERIFIED COMPLETE:</th>
<th>OSU PROTOCOL NUMBER:</th>
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1. **PROJECT TITLE**

Determination of the satiety index of two soft pretzels.

2. **INSTITUTIONAL REVIEW BOARD**

Select the Board to review this research:
- ☒ Behavioral and Social Sciences
- ☐ Biomedical Sciences
- ☐ Cancer

*Final Board assignment is determined by ORRP.*

3. **PRINCIPAL INVESTIGATOR (or Advisor) - see Qualifications for service as a PI**

<table>
<thead>
<tr>
<th>Name (Last, First, MI):</th>
<th>Vodovoz, Yael</th>
<th>Degree(s):</th>
<th>PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>University Academic Title:</td>
<td>Associate Professor</td>
<td>College (TIU):</td>
<td>Food, Agricultural, and Environmental Sciences</td>
</tr>
<tr>
<td>Department Name (TIU):</td>
<td>Food Science and Technology</td>
<td>Department # (TIU):</td>
<td>1156</td>
</tr>
<tr>
<td>Campus Mailing Address:</td>
<td>2015 Fyffe Ct</td>
<td>OSU ID Number:</td>
<td>00063063</td>
</tr>
<tr>
<td>Columbia, OH 43210</td>
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<tr>
<td>E-mail:</td>
<td><a href="mailto:vodovoz.1@osu.edu">vodovoz.1@osu.edu</a></td>
<td>Fax:</td>
<td>614-292-0218</td>
</tr>
<tr>
<td>Phone:</td>
<td>614-247-7696</td>
<td>Emergency phone:</td>
<td>614-570-4383</td>
</tr>
</tbody>
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4. **CO-INVESTIGATOR(S)**

Are there any OSU Co-Investigators on this protocol?
- ☒ Yes → Complete Appendix A1
- ☐ No

Signatures of Co-Investigator(s) are required on Appendix A1.

5. **KEY PERSONNEL**

Are there any OSU key personnel on this protocol?
- ☒ Yes → Complete Appendix A1
- ☐ No

Key personnel are defined as individuals who participate in the design, conduct, or reporting of human subjects research. At a minimum, include individuals who recruit or consent participants or who collect study data.

6. **EXTERNAL CO-INVESTIGATOR(S) & KEY PERSONNEL**

Are any external (non-OSU) Investigators or key personnel engaged in the OSU research?
- ☒ Yes
- ☐ No → Go to Question #7

*"Engaged" individuals are those who intervene or interact with participants in the context of the research or who will obtain individually identifiable private information for research funded, supervised, or coordinated by OSU. See [http://www.osu.edu/ohrp/human subjects/assurance/engage.htm](http://www.osu.edu/ohrp/human subjects/assurance/engage.htm) or contact ORRP for more information.*

If Yes → Who will provide approval for these external personnel?
- ☐ OSU IRB → Complete Appendix A2
- ☐ Non-OSU IRB → [Provide a copy of the approval(s)]
7. ADDITIONAL CONTACT(S) □ N/A
If further information about this application is needed, specify the contact person(s) if other than the PI (e.g., study or regulatory coordinator, research assistant, etc.).

<table>
<thead>
<tr>
<th>Name (Lnst. First, MI):</th>
<th>Simmons, Ambar L</th>
<th>Phone:</th>
<th>614-247-7686</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-mail:</td>
<td><a href="mailto:simmons.225@osu.edu">simmons.225@osu.edu</a></td>
<td>Fax:</td>
<td></td>
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All OSU individuals listed on this protocol will have access to information about IRB actions and the completion status of each individual's administrative and training requirements (CITI, COI disclosure). Note: Personal financial information provided in COI disclosures is not included.

8. EDUCATION
Have all OSU investigators and key personnel completed the required web-based course (CITI) in the protection of human research subjects?

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Educational requirements (initial and continuing) must be satisfied prior to submitting the application for IRB review. See http://orrp.osu.edu/irb/training/citi.cfm or contact ORRP for more information.

9. CONFLICT OF INTEREST
Does any OSU investigator (including principal or co-investigator), key personnel, or their immediate family members have a significant financial interest (e.g., speaking and consultation fees, travel expenses, proprietary interest in the tested product, stock ownership or other equity or membership in the sponsor over $10,000 per year or representing greater than 5% ownership in the sponsor) with the entity supporting the research or any company that may benefit from the research?

<table>
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<th>Yes</th>
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All OSU investigators and key personnel must have a current COI disclosure form filed before IRB review. See http://orcr.osu.edu/cei/index.cfm for more information.

10. FUNDING OR OTHER SUPPORT
a. Is the research funded or has funding been requested?

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If Yes → Specify sponsor: OARDC Seeds Grant and provide OSU RF project number:

b. Is there any support other than monetary (e.g., drugs, equipment, etc.) being provided for the study?

<table>
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<tr>
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If Yes → Specify: If the research is federally funded and involves a subcontract to or from another entity, an IRB Authorization Agreement may be required. Contact ORRP for more information.

11. OTHER INSTITUTIONAL APPROVALS
Check all that apply and provide applicable documentation. See websites listed below for information on obtaining approvals.

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<th>None</th>
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Clinical Research Center (CRC) Scientific Advisory Committee (SAC) – Approval required for research sponsored by the CRC. Final IRB approval will be held pending receipt of SAC approval. See www.crc.osu.edu.

Institutional Biosafety Committee (IBC) – Approval required for research involving bihazards (recombinant DNA, infectious or select agents, toxins), gene transfer, or xenotransplantation. See http://orrp.osu.edu/ibc/ or contact ORRP.

Comprehensive Cancer Center (CCC) Clinical Scientific Review Committee (CSRC) – Approval or exemption required for cancer-related research. See www.osuccc.osu.edu/cscc or contact the CCC Clinical Trials Office.

Maternal-Fetal Welfare Committee – Approval required for some research involving pregnant women and fetuses. See http://orrp.osu.edu/irb/osupolicies/MFReview.cfm or contact ORRP.
Human Subjects Radiation Committee (HSRC) – Approval required for research involving radiological procedures for research purposes (e.g., non-clinical care X-rays, DEXA or CT scans, nuclear medicine procedures, etc.). See www.ohio-state.edu or contact ORRP.

For the research described above, IRB review cannot be conducted until required institutional approvals or exemptions are obtained, except as noted.

12. LOCATION OF THE RESEARCH

a. List the specific site(s) at which the OSU research will be conducted (include both domestic and international locations).

<table>
<thead>
<tr>
<th>Location Name (or description)</th>
<th>Address (street, city and state, or country)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vodovotz Lab</td>
<td>240 Parker Food Science and Technology, 2015 Fyffe Ct., Columbus, OH 43210</td>
</tr>
</tbody>
</table>

b. Are all the sites named above on the OSU list of approved research performance sites? See http://orrp.osu.edu/irb/osupolicy/research/sites.cfm.

If No →
- Domestic sites → Provide a letter of support, as applicable
- International sites → Complete Appendix U

Research to be conducted at locations other than approved performance sites will minimally require a letter of support and may require another IRB’s approval if personnel are engaged. See http://www.hhs.gov/ohrp/humansubjects/assurance/engage.htm or contact ORRP for more information.

13. EXPEDITED REVIEW

Are you requesting Expedited Review?
- Yes → Complete Appendix B
- No

14. SUMMARY OF THE RESEARCH

Summarize the proposed research using non-technical language that can be readily understood by someone outside the discipline. Explain briefly the research design, procedures to be used, risks and anticipated benefits, and the importance of the knowledge that may reasonably be expected to result. Use complete sentences (limit 300 words).

The bulk of research regarding the nutritional benefits of soy foods has focused on the protection against cancer and heart disease, mainly via soy isoflavones and soy protein, respectively. However, due to the unique composition of soy foods they may beneficially impact postprandial glycemia and satiety, which may in turn help to reduce the risk of obesity and diabetes. A highly acceptable soy soft pretzel containing 8 g protein per serving has been developed at OSU and is being commercialized. The objective of this proposal is to determine the satiety index and the general acceptability of this soy pretzel and compared to conventional wheat soft pretzels in healthy adults. This study is being performed in conjunction with the investigation of the glycemic and insulin indexes of the soy soft pretzel compared to the wheat pretzel. Preliminary results from this study would lay the foundation for larger studies to examine the effects of daily soy intake on overall glycemic control in patients with diabetes and on long-term regulation of food intake for weight management.

15. SCIENTIFIC BACKGROUND & LITERATURE REVIEW

Summarize existing knowledge and previous work that support the expectation of obtaining useful results without undue risk to human subjects. Use complete sentences (limit 300 words).

Several studies have documented that a soy bread made with a patent pending technology and formulation developed at The Ohio State University had excellent consumer acceptability, improved shelf-life, and that its isoflavone content was bioavailable. However, in a short-term test market in the Columbus area, consumers expressed a desire for a snack like item with the same qualities. Therefore, a soy soft pretzel has been developed at a Cincinnati bakery using this technology and formulation. Preliminary results showed a highly acceptable product delivering 8 grams of soy protein per serving.

A 2004 review on high protein diets accumulated a great deal of data that, together, provide a very convincing argument that high
protein foods have a larger satiety index when compared to those that are mostly carbohydrates and fats (TL Hallton and FB Hu). Soy pretzels possess a higher percentage of protein (8 g vs. 5 g) in addition to more dietary fiber than wheat pretzels and are hypothesized to be more satiating. The effects of any soy-enhanced bakery formulation have not been determined and the effects of this formulation on postprandial satiety, specifically, have not been investigated. The proposed project will provide such information as well as insight into future improvements that could be made to the soft pretzel more acceptable and/or to help people maintain a healthy body weight.

16. RESEARCH OBJECTIVES

List the specific scientific or scholarly aims of the research study.

Our long-term goal is to develop new dietary strategies for the maintenance of healthy body weight and blood glucose levels, especially with regard to diabetes and pre-diabetes. The overall objective of this research is to determine if the soy pretzel can increase satiety relative to a conventional pretzel. The central hypothesis is that the soy pretzel will promote short-term satiety. The rationale for this hypothesis relies on previous studies of other protein-rich products as well as the unique nutritional profile of soybeans (e.g., energy density, protein content) that are known to influence satiety. We also want to assure that the soy-based soft pretzel is generally acceptable to consumers. We propose the following aims to address these objectives:

Aim 1: To determine the satiety index of soy pretzel and validate this subjective measure against measured food intake. The working hypothesis is that the soy pretzel will have a satiety index that is greater than that of a conventional pretzel. The subjective rating of satiety, based on questionnaires, will be used to calculate the satiety index of the soy and wheat soft pretzels.

Aim 2: To determine the acceptability of the soy and wheat soft pretzels using a 9-point hedonic scale.

17. RESEARCH METHODS & ACTIVITIES

a. Identify and describe all interventions and interactions that are to be performed solely for the research study. Distinguish research (i.e., experimental) activities from non-research activities.

Healthy people between the ages of 18 and 45 are able to volunteer for this study. It does not matter if the participant is obese, diabetic, a smoker, etc. as in our parallel glycemic index study. However, the candidate must not be allergic to wheat or soy. We will advertise by posting flyers around The Ohio State University campus. If a person is interested, they will contact Amber Simmons, the study coordinator, and she will set up a meeting with them to sign the consent form and proceed with the study. The participant will be invited to the Vodovoz lab (240 Parker Food Science and Technology) and be given the consent form which, if interested, he or she will sign. The participant will be able to ask questions about the study at any time. The age and the gender of the participant will be recorded so we can accurately report the ages of our participants and so that we can calculate the percentage of males and females. To avoid bias, the participant will be told that two pretzel recipes are being tested but they will not know that one is 25% soy and one is 100% wheat. After consent has been given, a small sample of both the wheat and the soy pretzels, labeled with a random 3-digit number, will be given to the participant in a random order. After they sample the first pretzel sample, they will rate the “acceptability” of the pretzel on the 9-point hedonic scale developed by David Perry (1957). A rating of 1 is “dislike extremely”, and a rating of 9 is “like extremely”. Water will be provided in order to cleanse their palate in between pretzel samples. After the second pretzel sample the second acceptability question will be answered. The participant will then be given a wheat pretzel and a soft pretzel both in plastic ziplock bags labeled clearly with “Day 1” or “Day 2”. Whether the soy pretzel or the wheat pretzel is “Day 1” will be random. In addition to the pretzels, the participant will also receive written instructions on how to complete the satiety testing. Instructions will also be given orally to assure that the participant understands the procedure. The participants will be told to store the pretzels in a cool, dark place like a cabinet (not the refrigerator) until they are eaten. The participant will fast for 10-12 h overnight. In the morning, the participant will eat the pretzel marked “Day 1”. They will describe how full the pretzel made them on a 9-point satiety scale anchored with “extremely hungry” on the left and “extremely full” on the right. They will declare their satiety (“fullness”) immediately after they eat the pretzel, 30 min after the first bite, 1 h after the first bite, and 2 hrs after the first bite. Within that time the participant should not eat any other foods or drink anything besides water. We will ask the participants to record how much water they drink within the two hours because this might affect their feeling of satiety. For the rest of the day they will be able to eat or drink whatever they want. He or she will repeat the overnight fast that night and the next morning he or she will repeat the procedure with the other pretzel. In summary, here is a time line:
When the participant finishes the study, he or she will bring the 2 completed questionnaires back to the Vodovotz lab where he or she will receive a $5 coupon to the Parker Dairy Store. For confidentiality, the acceptability and satiety questionnaires will be coded with a 2-digit number and will not include the participant's name.

The acceptability data will be a collection of numbers from 1 to 9 for the soy and the wheat pretzels. The numbers will be averaged to generate a single, mean acceptability rating for the soy and the wheat pretzels plus or minus a standard deviation. We hypothesize that the soy pretzel will be deemed acceptable with a mean acceptability rating between 5 and 9. We also hypothesize that the soy pretzel will have a lower acceptability rating than the wheat pretzel because the taste is a little different from what people are used to and different than their expectations of what a pretzel should taste like. We do not need to statistically compare the means, however.

The satiety data will be analyzed in a similar fashion. The mean satiety scores and their standard deviations will be calculated and the means will be compared using a two-sample, two-sided Student's t-test. Separate tests will be performed for each time point. We hypothesize that the soy pretzel will yield a higher satiety rating than the wheat pretzel and the differences in satiety will increase with time.

b. Check all research activities that apply:

- Anesthesia (general or local) or sedation
- Audio, video, digital, or image recordings
- Biohazards (e.g., rDNA, infectious agents, select agents, toxins)
- Biological sampling (other than blood)
- Blood drawing
- Coordinating Center
- Data, not publicly available
- Data, publicly available
- Data repositories (future unspecified use, including research databases)
- Deception (Complete Appendix D & Appendix MI)
- Devices (Complete Appendix E)
- Diet, exercise, or sleep modifications
- Drugs or biologics (Complete Appendix F)
- Emergency research
- Materials that may be considered sensitive, offensive, threatening, or degrading
- Non-invasive medical procedures (e.g., EKG, Doppler)
- Observation of participants (including field notes)
- Oral history (does not include medical history)
- Placebo
- Pregnancy testing
- Program Protocol (Umbrella Protocol)
- Radioisotopes or other sources of ionizing radiation
- Radioactive materials (requires approval from Radiation Safety Committee)
- Randomization
- Record review (which may include PHI)
- Specimen research
- Stem cell research
- Storage of biological materials (Complete Appendix H (future unspecified use, including repositories)
18. DURATION
Estimate the time required from each participant, including long-term follow-up, if any. Describe the time commitment in detail.

The participant will come to the Vodovoz lab and be given the consent form. If the person decides to participate he or she will sign the consent form. The participant will be able to ask questions about the study at any time. After consent has been given, a small sample of both the wheat and the soy pretzel will be given to the participant in a random order. They will rate the “acceptability” of both pretzels on the 9-point hedonic scale. Then, the participant will be given a soy pretzel and a wheat pretzel with instructions on how to do the satiety tests. This meeting will last approximately 46 min; 20 min to read and sign the consent form, 10 min to perform the acceptability test, and 10 min to explain the satiety test. That night, the participant will fast for 16-12 hrs overnight. In the morning, the participant will eat the first pretzel, as was assigned to them. This will take about 10 min. They will then describe how full the pretzel made them. This will take about 10 sec. They will declare their satiety (“fullness”) immediately after they eat the pretzel, 30 min after the first bite, 1 hr after the first bite, and 2 hrs after the first bite. Within that time the participant should not eat any other foods or drink anything besides water. For the rest of the day they will be able to eat or drink whatever they want. He or she will repeat the overnight fast that night and the next morning he or she will repeat the procedure with the other pretzel. In summary, the participant will be involved for 3 days. On day 0, the participant will perform the acceptability test at the Vodovoz lab and pick up the pretzels for the satiety test. On day 1 he or she will perform one satiety test and on day 2 he or she will perform the second satiety test. The satiety questionnaires can be returned at their convenience but preferably as soon as possible.

19. NUMBER OF PARTICIPANTS
a. Provide the maximum number of participants (or number of participant records, specimens, etc.) for whom you are seeking OSU IRB approval.

The number of participants is defined as the number of individuals who agree to participate (i.e., those who provide consent or whose records are accessed, etc.) even if all do not complete the study. The proposed maximum should include the number of participants who are required (considering participation criteria, withdrawals, etc.) to obtain the desired outcome of the study.

b. Explain how this number was derived.

Question: Does the soy pretzel have a different satiety index than the wheat pretzel?
The data will be analyzed with a two-sided, two-sample t-test with α=0.05 and 90% power.

δ = satiety(soy) – satiety(wheat)

H₀: δ = 0  There is no difference in satiety index between wheat and soy pretzels
H₁: δ ≠ 0  There is a difference in satiety index between wheat and soy pretzels

Define margin of error, m = 1

Estimate standard deviation, s = 3

\[
m = t_{n-1} \left(1 - \frac{\alpha}{2}\right) \frac{s}{\sqrt{n}}
\]

\[
1 = t_{40}(0.975) \frac{s}{\sqrt{n}}
\]

n = 37

We need an n of 37 to distinguish a difference in the satiety indexes of the wheat and soy pretzel. In case compliance is not 100%, we would like to recruit 50 participants.
c. Is this a multi-center study? ☐ Yes → Indicate the total number of participants to be enrolled across all sites: ______________
☐ No

The total number of research participants may be increased only with prior IRB approval.

20. PARTICIPANT POPULATION

a. Specify the age(s) of the individuals who may participate in the research:

Age(s): 18-45

b. Specify the participant population(s) to be included (check all that apply):

☐ Adults
☐ Adults unable to consent for themselves
☐ Children (< 18 years) → Complete Appendix I
☐ Healthy volunteers
☐ Neonates (uncertain viability/noviable) → Complete Appendix K
☐ Non-English speaking → Complete Appendix J
☐ Pregnant Women/Fetuses → Complete Appendix K
☐ Prisoners → Complete Appendix J
☐ Students from participant pools (e.g., REP)
☐ Unknown (e.g., research using secondary data/specimens, non-targeted surveys, program protocols)

Specify:

We will be studying healthy individuals between the ages of 18 and 45 recruited from a college campus. We will be recruiting healthy people (as opposed to those with diabetes or glucose intolerance) for this a preliminary study. Moreover, this population will most likely comprise a large proportion of the soy soft pretzel consumers once the pretzels are commercialized. The only exclusion criteria are that the person cannot be allergic to wheat or soy or be pregnant.

c. Describe the characteristics of the population(s) and explain how the nature of the research requires/justifies inclusion of the proposed population(s).

We will be studying healthy individuals between the ages of 18 and 45 recruited from a college campus. We will be recruiting healthy people (as opposed to those with diabetes or glucose intolerance) for this a preliminary study. Moreover, this population will most likely comprise a large proportion of the soy soft pretzel consumers once the pretzels are commercialized. The only exclusion criteria are that the person cannot be allergic to wheat or soy or be pregnant.

d. Will pregnant women be excluded from participation in the research? ☐ Yes
☐ No

If Yes → Explain how the nature of the research requires/justifies their exclusion. Address means of pregnancy screening. Pregnant women will be excluded due to potential effects on glucose metabolism and requirements for fasting. A simple yes or no question will determine whether a participant is pregnant or not.

21. PARTICIPANT IDENTIFICATION, RECRUITMENT, & SELECTION

a. Describe how potential participants will be identified (e.g., advertising, individuals known to investigator, record review, etc.).

Explain how the method(s) for identifying potential participants respects their privacy.

Flyers will be displayed around The Ohio State University campus. The flyer will include the study coordinator’s email address so that interested people can contact her regarding the study. To respect the privacy of the participants, all acceptability and safety questionnaires will be labeled with the participant’s 2-digit number, not his or her name. The sheet matching each individual to their corresponding number will be discarded as soon as all raw data is obtained and will not be used for analysis or any other purpose. The study coordinator will keep all data in a locked file cabinet and all electronic data will be password protected. Raw data will not be distributed; only analyzed data will be reported and published.

b. State who (investigators and/or key personnel) will recruit participants and what process will be used to determine participant eligibility.
The study coordinator will be in charge of recruiting individuals. Flyers will be posted around Ohio State campus stating the eligibility criteria: age 18-45, not pregnant, and not allergic to wheat or soy. Interested people can email the study coordinator. All questions will be answered via email or in person. We will simply ask the candidate if she is pregnant, if applicable, or if he or she has any wheat or soy allergies. If the person doesn’t know if he or she is allergic to soy we will discourage them from participating but not deem them ineligible.

c. Describe the recruitment process; including how and where recruitment will take place. Provide copies of proposed recruitment materials (e.g., ads, flyers, website postings, recruitment letters, and oral/written scripts). Recruitment will begin as soon as the IRB is approved. Flyers (see attached) will be posted around the Ohio State University campus and subjects will be asked to contact the study coordinator, Amber Simmons, if interested. Amber will discuss the details of the study via email and, if the person is interested, set up a meeting for him or her to come in. At the meeting, Amber will provide the participant with a consent form. The participant will read it and ask any questions that he or she may have. After the person signs the consent form we will proceed with the study.

22. INCENTIVES TO PARTICIPATE

Will participants receive compensation or other incentives (e.g., free services, cash payments, gift certificates, parking, classroom credit, travel reimbursement) to participate in the research study?  
☐ Yes  ☐ No

If Yes → Describe the incentive. Compensation should be pro-rated (e.g., per visit) and not contingent upon study completion.

When the participant submits the 2 completed satisfaction forms he or she will be given a $5 coupon to the Parker Dairy Store in Parker Food Science and Technology, 2015 Fyffe Ct. Even if a person drops out of the study they will be able to receive the $5 coupon to the Dairy Store.

23. INFORMED CONSENT PROCESS

a. Indicate the consent process(es) and document(s) to be used in the study. Check all that apply.

☐ Assent – Form
☐ Assent – Verbal Script
☐ Informed Consent – Form
☐ Informed Consent – Verbal Script

☐ Parental Permission – Form
☐ Parental Permission – Verbal Script
☐ Translated Consent/Assent – Form(s)
☐ Waiver or Alteration of Consent Process

Provide copies of documents (using OSU templates) and/or complete relevant appendices, as needed. See http://orrp.osu.edu/irb/consent/index.cfm or contact ORRP for more information.

b. Describe the consent process. Explain when and where consent will be obtained and how subjects and/or their legally authorized representatives will be provided sufficient opportunity (e.g., waiting period, if any) to consider participation.

Before the meeting with the participant, the study will be explained individually to the participants via email. If a person expresses interest, he or she will be invited to the Vodovotz lab for the acceptability test and to pick up supplies. At the meeting, the first thing the subject will do is read the informed consent. At any time the participant will be allowed to ask questions. Informed consent will thus be obtained before any eating or filling out of questionnaires. The consent form will be approved by the Institutional Review Board and will detail the purpose of the study, the procedure, the nature of the intervention, risks and benefits, confidentiality, and options for withdrawal from the study. Each subject will be informed both verbally and with the written consent document about the benefits and risks associated in participating in the study, and all questions concerning the study will be answered.

c. List the investigator(s) and/or key personnel who will obtain consent from participants or their legally authorized representatives.  
☐ N/A
Amber Simmons

d. Explain how the possibility of coercion or undue influence will be minimized in the consent process.  N/A

All of the participant’s questions will be answered thoroughly and truthfully. It will be explained that each subject has the choice not to participate and will be able to leave the study at any time without stating a reason for withdrawal. The amount of compensation for participation in this study was chosen on the basis of being fair amount without being so high that it might be a significant reason to participate in the study.

e. Will any other tools (e.g., quizzes, visual aids, information sheets) be used during the consent process to assist participant comprehension?  Yes → Provide copies of these tools

f. Will any other consent forms be used (e.g., for clinical procedures such as MRI, surgery, etc. and/or consent forms from other institutions)?  Yes → Provide copies of these forms

<table>
<thead>
<tr>
<th>24. CAPACITY TO CONSENT</th>
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</table>
| Will adult participants with limited decision-making capacity or who lack the ability to consent be recruited in this research study?  Yes

If Yes → Describe the likely range of participant impairment and explain how, and by whom, the capacity to consent/assent will be determined. For adults unable to provide legally effective informed consent, indicate whether assent will be obtained; or if not, explain why not.

<table>
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<tr>
<th>25. PRIVACY &amp; CONFIDENTIALITY</th>
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| Does the research require access to personally identifiable private information?  Yes

If Yes → Describe the steps you will take to ensure protection of the participants’ privacy.

b. Will personal or sensitive information (e.g., relating to illegal behaviors, alcohol or drug use, sexual attitudes, mental health, etc.) be accessed or collected from participants?  Yes

If Yes → Describe information.

c. Could disclosure of information be potentially damaging to participants’ financial standing, employability or reputation, or place the participants at risk of criminal or civil liability?  Yes

If Yes → Explain.

d. Explain how you will protect the confidentiality of identifiable data, including where data will be stored, what security measures will be applied, and who will have access to the data.

Only the principle investigator and the study coordinator will have access to the questionnaires. The questionnaires will be coded and not labeled with personal data. Data that is entered into a computer will be password protected.

e. Will you be obtaining a NIH Certificate of Confidentiality?  Yes → Provide a copy before you begin the research

f. Explain any circumstances (ethical or legal) where it would be necessary to break confidentiality.  ☒ N/A

g. Indicate what will happen to the identifiable data at the end of the study. Check all that apply:
   ☐ Identifiers separated or permanently removed from the data
   ☒ Identifiable-coded data is retained
   ☐ Other, specify: ____________________________
   ☐ N/A

h. Indicate how study results might be disseminated. Check all that apply:
   ☒ Conference/Presentation
   ☒ Dissertation/Thesis
   ☒ Publication/journal article
   ☐ Other, specify: ____________________________

26. HIPAA RESEARCH AUTHORIZATION

Will individually identifiable Protected Health Information (PHI) subject to the [HIPAA Privacy Rule](https://www.hhs.gov/hipaa) requirements be accessed, used, or disclosed in the research study?  ☐ Yes  ☒ No  Go to Question #27

If Yes  → Will a written authorization be used?

   ☐ Yes  → Provide a copy of the Authorization Form

   a. Describe the PHI involved in the research (e.g., demographic information, health history, diagnosis, test results). Be as specific as possible. Provide a copy of the data collection form(s) to be used.

   b. List the source(s) of the PHI (e.g., OSUMC Information Warehouse, physician’s own records, etc.), including whether any information will be obtained from sources external to OSU.

   ☐ No  → Indicate the type of waiver or alteration requested (check all that apply) and complete Appendix N.

      ☐ Partial Waiver (recruitment purposes only)
      ☐ Full Waiver (entire research study)
      ☐ Alteration (written documentation)

27. REASONABLY ANTICIPATED BENEFITS

a. List the potential benefits that participants may expect as a result of this research study. State if there are no direct benefits to individual participants. Compensation is not to be considered a benefit. There is no direct benefit to the participants of the study.

b. List the potential benefits that society and/or others may expect as a result of this research study.

Society will gain knowledge of the satiety index of acry-based baked goods compared to wheat-based baked goods. This is especially important for the diabetic community and anyone who is trying to lose weight by eating less.
28. RISKS, HARMS, & DISCOMFORTS

a. Indicate all reasonably expected risks/harms/discomforts that may apply to the research study:

- [ ] Breach of confidentiality
- [ ] Psychological stress
- [x] Discovery of previously unknown condition
- [ ] Risk to reputation
- [ ] Economic risk
- [ ] Social or legal risk
- [ ] Invasion of privacy (participants or other individuals)
- [ ] Other
- [ ] Physical injury or discomfort
- [ ] Specify:

b. For each category of risk checked above, describe the specific risk. For physical injury or discomfort include the following:

- Frequency/likelihood of occurrence
- Potential severity of the harm/discomfort
- Possible consequences (including long-term effects)

Reference the section of this application (e.g., Appendix F for drugs) if the risks are described elsewhere.

It is possible, although rare, for a participant to have never encountered soy before and be allergic. If an allergic reaction occurs during the acceptability test the person will not proceed with the satiety test. If an allergic reaction occurs during the satiety test the person will be encouraged to seek help immediately and not complete the satiety survey. About 1% of the population has soy allergies and most are diagnosed when the person is still an infant or a small child. In most cases, allergic reactions to soy are mild and include redness, rash, inflammation, or hives but can be as severe as anaphylactic shock.

c. Describe the specific protections that will be used to minimize the identified risks and harms.

The person will be asked directly if they have had soy products before (soy sauce counts) to assure that they are not allergic to soy. This will occur in the preliminary emails, before the meeting is set up. If they do not know, the study coordinator will discourage participation.

29. MONITORING

Does the research involve greater than minimal risk (i.e., are the harms or discomforts described in Question #28 beyond what is ordinarily encountered in daily life or during the performance of routine physical or psychological tests)?

- [ ] Yes
- [x] No

If Yes → Describe the plan to oversee and monitor data collected to ensure participant safety and data integrity. Include the following:

- The information that will be evaluated (e.g., incidence and severity of actual harm compared to that expected);
- Who will perform the monitoring (e.g., investigator, sponsor, or independent monitoring committee);
- Timing of monitoring (e.g., at specific points in time, after a specific number of participants have been enrolled); and
- Decisions to be made as a result of the monitoring process (e.g., provisions to stop the study early for unanticipated problems).

30. ASSESSMENT OF RISKS & BENEFITS

Discuss how risks to participants are reasonable when compared to the anticipated benefits to participants (if any) and the importance of the knowledge that may reasonably be expected to result.

Risk for the participants will be minimal, and the participants will be compensated with a $5 coupon to the Parker Dairy Store. Most likely, participants will not have tried a soy-based baked good before and will be granted this special opportunity. We will closely monitor every participant and will immediately discontinue the study if we see any signs of toxicity or allergic reactions. Knowledge gained from this study will supplement the currently limited knowledge pool on soy products. It could potentially change how dieters view soy-enhanced products and could potentially influence the direction of food companies that heed the satiety index.
31. ALTERNATIVES TO STUDY PARTICIPATION

Other than choosing not to participate, list any specific alternatives, including available procedures or treatments that may be advantageous to the subject.

There are no alternatives to participation.

32. PARTICIPANT COSTS/REIMBURSEMENTS

a. List any potential costs subjects (or their insurers) will incur as a result of study participation (e.g., parking, study drugs, diagnostic tests, etc.).

The participant may need to pay for parking. Most participants, however, are expected to be students and can walk or take the CABS bus from campus.

b. List any costs to participants that will be covered by the research study.

The cost to produce and distribute the pretzels, the time and effort of the study coordinators, and all future data analyses will be covered by the research study.
33. APPLICATION CONTENTS

Indicate what documents are being submitted for this research project. Check all appropriate boxes and provide the version number and date, if available.

- Initial Review of Human Subjects Research Application
  - Version: 2.0
  - Date: 10/6/08
- Appendix A1: OSU Co-Investigators & Key Personnel (questions 4 & 5)
  - Version: 2.0
  - Date: 10/6/08
- Appendix A2: External (non-OSU) Co-Investigators & Key Personnel (question 6)
- Appendix B: Expedited Review – Initial Review (question 13)
  - Version: 2.0
  - Date: 10/6/08
- Appendix C: Data Repositories (question 17b)
- Appendix D: Deception (question 17b)
- Appendix E: Devices (question 17b)
- Appendix F: Drugs or Biologies (question 17b)
- Appendix G: Generic Testing (question 17b)
- Appendix H: Storage of Biological Materials (question 17b)
- Appendix I: Children (question 20b)
- Appendix J: Non-English Speaking Participants (questions 20b and 23a)
- Appendix K: Pregnant Women/Tetuses/Neonates (question 20b)
- Appendix L: Prisoners (question 20b)
- Appendix M1: Waiver or Alteration of Consent Process (questions 17b & 23a)
- Appendix M2: Waiver of Consent Documentation (question 23a)
- Appendix N: Waiver of HIPAA Research Authorization (question 26)
- Appendix U: Research in International Settings (question 12)
- Consent Form(s), Assent Form(s), Permission Form(s), and Verbal Script(s), including translated documents (question 23a)
- HIPAA Research Authorization Form(s) (question 26)
- Data Collection Form(s) involving protected health information (question 20a)
- Recruitment Materials (e.g., ads, flyers, telephone or other oral script, radio/TV scripts, internet solicitations) (question 21c)
- Script(s) or Information Sheet(s), including Debriefing Materials (question 23a)
- Instruments (e.g., questionnaires or surveys to be completed by participants) (question 17b)
- Other Committee Approvals/Letters of Support (questions 11 & 12)
- Research Protocol
- Complete Grant Application
- Drug Manufacturer’s Approved Labeling/Investigator’s Drug Brochure (Appendix F)
- Device Manufacturer’s Approved Labeling (Appendix E)
- Other supporting documentation and/or materials

For Multi-Center Clinical Trials supported by DHHS, the submission will also include:
- DHHS-approved Sample Informed Consent Document (if one exists)
- DHHS-approved Protocol (if one exists)

34. ASSURANCE

PRINCIPAL INVESTIGATOR (or Advisor)
I agree to follow all applicable policies and procedures of The Ohio State University and federal, state, and local laws and guidance regarding the protection of human subjects in research, as well as with professional practice standards and generally accepted good research practice guidelines for investigators, including, but not limited to, the following:

- The research will be performed as approved by the IRB under the direction of the Principal Investigator (or Advisor) by appropriately trained and qualified personnel with adequate resources;
- The research will not be initiated until written notification of IRB approval has been received;
- Informed consent and HIPAA research authorization from human subjects (or their legally authorized representatives) will be obtained and documented (unless waived) prior to their involvement in the research using the currently IRB-approved consent form(s) and process;
- Promptly report to the IRB events that may represent unanticipated problems involving risks to subjects or others;
- Significant new findings that develop during the course of the study that may affect the risks or benefits of participation will be reported;
- The IRB will be informed of any proposed changes in the research or informed consent process before changes are implemented, and no changes will be made until approved by the OSU IRB (except where necessary to eliminate apparent immediate hazards to participants);
- A Continuing Review of Human Subjects Research application will be completed and submitted before the deadline for review at intervals determined by the IRB to be appropriate to the degree of risk (but not less than once per year) to avoid expiration of IRB approval and cessation of all research activities;
- Research-related records (and source documents) will be maintained in a manner that documents the validity of the research and integrity of the data collected, while protecting the confidentiality of the data and privacy of participants;
- Research-related records will be retained and available for audit for a period of at least three years after the research has ended (or longer, according to sponsor or publication requirements) even if I leave the University;
- The Office of Responsible Research Practices will be contacted for assistance in amending (to request a change in Principal Investigator) or terminating the research if I leave the University or am unavailable to conduct or supervise the research personally (e.g., sabbatical or extended leave);
- A Final Study Report will be provided to the IRB when all research activities have ended (including data analysis with individually identifiable or coded private information); and
- All Co-Investigators, research staff, employees, and students assisting in the conduct of the research will be informed of their obligations in meeting the above commitments.

I verify that the information provided in this Initial Review of Human Subjects Research application is accurate and complete.

Signature of Principal Investigator (or Advisor)  Date

Printed name of Principal Investigator (or Advisor)

DEPARTMENT CHAIR (or Signatory Official)

As Department Chair (or Signatory Official) for the Principal Investigator, I acknowledge that this research is in keeping with the standards set by our unit and that it has met all Departmental/College requirements for review.

If the PI or any Co-Investigator is also the Department Chair, the signature of the Dean or other appropriate Signatory Official, such as the Associate Dean for Research, must be obtained.

Signature of Department Chair  Date

Printed name of Department Chair
Appendix B: Consent form for Acceptability and Preliminary Satiety Study

The Ohio State University Consent to Participate in Research

Study Title: Determination of the Satiety Index of Two Soft Pretzels
Researcher: Yael Vodovotz, PhD
Sponsor: Ohio Agricultural Research and Development Center

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate.

Your participation is voluntary.

Purpose: Foods that have a high satiety index will fill you up more and result in less food consumed total. These foods can help prevent and treat obesity. We are developing a more healthful soft pretzel and are investigating whether either of these pretzel varieties is more filling than the other.

Procedures/Tasks: You will eat the two pretzels for breakfast on two consecutive days. After eating the pretzel you will fill out a questionnaire describing how full the pretzel made you. You will report your satiety again one hour after you eat the pretzel and two hours after you eat the pretzel. You may consume nothing besides water for these two hours.

Duration: The study will last 3 days, including today.

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

Risks and Benefits: The risks of this study are extremely low unless you are allergic to wheat or soy. If you have not had soy before you are discouraged from participating but not disallowed.

Confidentiality: Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information
regarding your participation in this study may be disclosed if required by state law. Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor, if any, or agency (including the Food and Drug Administration for FDA-regulated research) supporting the study.

**Incentives:** When you bring back the questionnaires you will receive a $5 coupon to the Parker Dairy Store. If the questionnaires are not complete because you decided to discontinue the study you will still receive the $5 coupon.

**Participant Rights:**

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

**Contacts and Questions:**

For questions, concerns, or complaints about the study you may contact Amber Simmons, the study coordinator, at simmons.225@osu.edu.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Yael Vodovotz at vodovotz.1@osu.edu.
CONSENT
Behavioral/Social Science

Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

<table>
<thead>
<tr>
<th>Printed name of subject</th>
<th>Signature of subject</th>
<th>AM/PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Date and time</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Printed name of person authorized to consent for subject (when applicable)</th>
<th>Signature of person authorized to consent for subject (when applicable)</th>
<th>AM/PM</th>
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<tbody>
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<td>Date and time</td>
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</table>

Relationship to the subject

<table>
<thead>
<tr>
<th>Printed name of person obtaining consent</th>
<th>Signature of person obtaining consent</th>
<th>AM/PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Date and time</td>
</tr>
</tbody>
</table>

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

<table>
<thead>
<tr>
<th>Printed name of person obtaining consent</th>
<th>Signature of person obtaining consent</th>
<th>AM/PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Date and time</td>
</tr>
</tbody>
</table>
# Appendix C: Acceptability Questionnaire

**Soft Pretzel Acceptability Questionnaire**

Please rate how much you like each sample. Please make sure you match the three-digit code on the sample to the scale below.

**Soft pretzel #302**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extremely dislike</td>
<td>Very much dislike</td>
<td>Moderately dislike</td>
<td>Slightly dislike</td>
<td>Neither like nor dislike</td>
<td>Slightly like</td>
<td>Moderately like</td>
<td>Very much like</td>
<td>Extremely like</td>
</tr>
</tbody>
</table>

**Soft pretzel #250**

<table>
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<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extremely dislike</td>
<td>Very much dislike</td>
<td>Moderately dislike</td>
<td>Slightly dislike</td>
<td>Neither like nor dislike</td>
<td>Slightly like</td>
<td>Moderately like</td>
<td>Very much like</td>
<td>Extremely like</td>
</tr>
</tbody>
</table>
Appendix D: Ohio State University Institutional Review Board

Acceptability and Initial Satiety Protocol

Exempt Approval
April 13, 2009

Protocol Number: 2009EO310
Protocol Title: DETERMINATION OF THE SATIETY INDEX OF TWO SOFT PRETZELS, Yael Vodovotz, Carla Miller, Amber Simmons, Food Science and Technology
Type of Review: Request for Exempt Determination
ORRP Staff Contact: Cheri M. Petey
Phone: 614-688-0189
Email: petey.6@osu.edu

Dear Dr. Vodovotz,

The Office of Responsible Research Practices has determined the above referenced protocol exempt from IRB review.

Date of Exempt Determination: April 13, 2009
Qualifying Exemption Category: 6

Please note the following:

- Only OSU employees and students who have completed CITI training and are named on the signature page of the application are approved as OSU Investigators in conducting this study.
- No procedural changes may be made in exempt research (e.g., recruitment procedures, advertisements, instruments, enrollment numbers, etc.).
- Per university requirements, all research-related records (including signed consent forms) must be retained and available for audit for a period of at least three years after the research has ended.
- It is the responsibility of the Investigator to promptly report events that may represent unanticipated problems involving risks to subjects or others.

This determination is issued under The Ohio State University’s OHRP Federalwide Assurance #00006378.

All forms and procedures can be found on the ORRP website — www.orrp.osu.edu. Please feel free to contact the ORRP staff contact listed above with any questions or concerns.

Cheri Petey, MA, Certified IRB Professional
Senior Protocol Analyst—Exempt Research
Appendix E: Ohio State University Institutional Review Board Application
for Satiety Study
INITIAL REVIEW OF HUMAN SUBJECTS RESEARCH
The Ohio State University Institutional Review Boards
Office of Responsible Research Practices (ORRP)
300 Research Administration Building, 1960 Kenny Road, Columbus, OH 43210
Phone: (614) 688-8457 Fax: (614) 688-0366 www.orrp.osu.edu

<table>
<thead>
<tr>
<th>SPEC</th>
<th>DATE RECEIVED:</th>
<th>DATE VERIFIED COMPLETE:</th>
<th>OSU PROTOCOL NUMBER:</th>
</tr>
</thead>
</table>

1. PROJECT TITLE

Determination of the saliety of two soft pretzels.

2. INSTITUTIONAL REVIEW BOARD

Select the Board to review this research:

- [ ] Behavioral and Social Sciences
- [ ] Biomedical Sciences
- [ ] Cancer

Final Board assignment is determined by ORRP.

3. PRINCIPAL INVESTIGATOR (or Advisor) - see Qualifications for service as a PI

Name (Last, First, MI): Vodovotz, Yael
Degree(s): PhD
University Academic Title: Associate Professor
College (ITU): Food, Agricultural, and Environmental Sciences
Department Name (ITU): Food Science and Technology
Department # (ITU): 1156
Campus Mailing Address: 2015 Fyffe Ct.
Columbus, OH 43210
OSU ID Number: 00093663
E-mail: vodovotz.1@osu.edu Fax: 614-292-0318
Phone: 614-292-7696 Emergency phone: 614-570-4303

4. CO-INVESTIGATOR(S)

Are there any OSU Co-Investigators on this protocol?
- [x] Yes ➔ Complete Appendix A1
- [ ] No

Signatures of Co-Investigator(s) are required on Appendix A1.

5. KEY PERSONNEL

Are there any OSU key personnel on this protocol?
- [x] Yes ➔ Complete Appendix A1
- [ ] No

Key personnel are defined as individuals who participate in the design, conduct, or reporting of human subjects research. At a minimum, include individuals who recruit or consent participants or who collect study data.

6.EXTERNAL CO-INVESTIGATOR(S) & KEY PERSONNEL

Are any external (non-OSU) Investigators or key personnel engaged in the OSU research?
- [ ] Yes
- [x] No ➔ Go to Question #7

“Engaged” individuals are those who intervene or interact with participants in the context of the research or who will obtain individually identifiable private information for research funded, supervised, or coordinated by OSU. See OHRP Engagement Guidance or contact ORRP for more information.

If Yes ➔ Who will provide approval for these external personnel?
- [ ] OSU IRB ➔ Complete Appendix A2
- [ ] Non-OSU IRB ➔ Provide a copy of the approval(s)
7. ADDITIONAL CONTACT(S)

If further information about this application is needed, specify the contact person(s) if other than the PI (e.g., study or regulatory coordinator, research assistant, etc.).

<table>
<thead>
<tr>
<th>Name (Last, First, MI):</th>
<th>Simmons, Amber L</th>
<th>Phone:</th>
<th>614-247-7686</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-mail:</td>
<td><a href="mailto:simmons.225@osu.edu">simmons.225@osu.edu</a></td>
<td>Fax:</td>
<td></td>
</tr>
</tbody>
</table>

All OSU individuals listed on this protocol will have access to information about IRB actions and the completion status of each individual’s administrative and training requirements (CITI, COI disclosure). Note: Personal financial information provided in COI disclosures is not included.

8. EDUCATION

Have all OSU investigators and key personnel completed the required web-based course (CITI) in the protection of human research subjects?

☑ Yes
☐ No

Educational requirements (initial and continuing) must be satisfied prior to submitting the application for IRB review. See CITI Training or contact ORRP for more information.

9. FINANCIAL CONFLICT OF INTEREST

Does any OSU investigator (including principal or co-investigator), key personnel, or their immediate family members have a financial interest (including salary or other payments for services, equity interests, or intellectual property rights) that would reasonably appear to be affected by the research, or a financial interest in any entity whose financial interest would reasonably appear to be affected by the research?

☑ Yes
☐ No

All OSU investigators and key personnel must have a current COI disclosure form (updated as necessary for the proposed research) filed before IRB review. Examples of financial interests that must be disclosed include (but are not limited to) consulting fees or honoraria; stocks, stock options or other ownership interests; and patents, copyrights and royalties from such rights. For more information, see Office of Research Compliance COI Overview and COI Forms.

10. FUNDING OR OTHER SUPPORT

If the research is federally funded and involves a subcontract to or from another entity, an IRB Authorization Agreement may be required. Contact ORRP for more information.

a. Is the research funded or has funding been requested?

☑ Yes
☐ No

If Yes ➔ Specify sponsor: OARDC Seeds Grant

Provide a copy of the grant application or funding proposal. The University is required to verify that all funding proposals and grants (new or renewals) have been reviewed by the IRB before funds are awarded.

b. Is any support other than monetary (e.g., drugs, equipment, etc.) being provided for the study?

☑ Yes
☐ No

If Yes ➔ Specify support and provider:

11. OTHER INSTITUTIONAL APPROVALS

Check all that apply and provide applicable documentation. See websites listed below for information on obtaining approvals. IRB review cannot be conducted until required institutional approvals or exemptions are obtained, except as noted.

☑ None
☐ Clinical Research Center (CRC) Scientific Advisory Committee (SAC) – Approval required for research sponsored by the
The Ohio State University Institutional Review Boards - INITIAL REVIEW OF HUMAN SUBJECTS RESEARCH

CRC. Final IRB approval will be held pending receipt of SAC approval.

☐ Institutional Biosafety Committee (IBC) – Approval required for research involving biohazards (recombinant DNA, infectious or select agents, toxins), gene transfer, or xenotransplantation.

☐ Comprehensive Cancer Center (CCC) Clinical Scientific Review Committee (CSRC) – Approval or exemption required for cancer-related research.

☐ Maternal-Fetal Welfare Committee – Approval required for some research involving pregnant women and fetuses.

☐ Human Subject Radiation Committee (HSRC) – Approval required for research involving radiologic procedures for research purposes (e.g., non-clinical care X-rays, DEXA or CT scans, nuclear medicine procedures, etc.).

12. LOCATION OF THE RESEARCH

Research to be conducted at locations other than approved performance sites will minimally require a letter of support and may require another IRB’s approval if personnel are engaged. See OHIRP Engagement Guidance or contact ORRP for more information.

a. List the specific site(s) at which the OSU research will be conducted (include both domestic and international locations).

<table>
<thead>
<tr>
<th>Location Name (or description)</th>
<th>Address (street, city and state, or country)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vodovotz Lab</td>
<td>240 Parker Food Science and Technology, 2015 Fyffe Ct., Columbus, OH 43210</td>
</tr>
</tbody>
</table>

b. Are all the sites named above on the OSU list of approved research performance sites? ☒ Yes → Go to Question #13

☐ No

If No → ☐ Domestic sites → Provide a letter of support, as applicable

☐ International sites → Complete Appendix U

c. For multi-site research, is the OSU PI the lead investigator or is OSU the lead site? ☐ Yes

☐ No → Go to Question #13

☐ Not multi-site → Go to Question #13

i. Describe the communication between sites that might be relevant to the protection of participants, such as unanticipated problems, interim results, and protocol modifications.

ii. Describe IRB oversight arrangements for each site (i.e., who is providing IRB review and approval). Provide copies of the non-OSU approvals, as applicable. Contact ORRP if requesting OSU be the IRB of record.

13. EXPEDITED REVIEW

Are you requesting Expedited Review? ☒ Yes → Complete Appendix B

☐ No

14. SUMMARY OF THE RESEARCH

Summarize the proposed research using non-technical language that can be readily understood by someone outside the discipline. Explain briefly the research design, procedures to be used, risks and anticipated benefits, and the importance of the knowledge that may reasonably be expected to result. Use complete sentences (limit 300 words).
People in developed countries are eating more frequently now than ever, and this change in behavior is correlated with an increase in the prevalence of obesity. There is a need for snack foods that provide essential nutrients and high feelings of satiety, helping people to prevent overeating. A highly acceptable soy soft pretzel containing 12.5 g protein per serving (238 kcal) has been developed at OSU and is being commercialized. The objective of this proposal is to determine the relative satiety level of this soy pretzel compared to conventional wheat soft pretzels in healthy adults. This study is being performed in conjunction with the investigation of the glycemic and insulin indexes of the soy soft pretzel compared to the wheat pretzel. Preliminary results from this study would lay the foundation for larger studies to examine the effects of daily soy intake on overall glycemic control in patients with diabetes and on long-term regulation of food intake for weight management.

15. SCIENTIFIC BACKGROUND & LITERATURE REVIEW

Summarize existing knowledge and previous work that support the expectation of obtaining useful results without undue risk to human subjects. Use complete sentences (limit 300 words).

Several studies have documented that a soy bread made with a patent pending technology and formulation developed at The Ohio State University had excellent consumer acceptability and improved shelf-life. However, in a short-term test market in the Columbus area, consumers expressed a desire for a snack like item with the same qualities. Therefore, a soy soft pretzel has been developed at a Cincinnati bakery using this technology and formulation. Preliminary results showed a highly acceptable product delivering 12.5 grams of soy protein per serving.

A 2004 review on high protein diets accumulated a great deal of data that, together, provide a very convincing argument that high protein foods have a larger satiety index when compared to those that are mostly carbohydrates and fats (TL Hallon and FB Hu). Soy pretzels possess a higher percentage of protein (12.5 g vs. 7.2 g) in addition to more dietary fiber than wheat pretzels and are hypothesized to be more satiating. The effects of any soy-enhanced bakery formulation have not been determined and the effects of this formulation on postprandial satiety, specifically, have not been investigated. The proposed project will provide such information as well as insight into future improvements that could be made to the soft pretzel more acceptable and/or to help people maintain a healthy body weight.

16. RESEARCH OBJECTIVES

List the specific scientific or scholarly aims of the research study.

Our long term goal is to develop new dietary strategies for the maintenance of healthy body weight and blood glucose levels, especially with regard to diabetes and pre-diabetes. The overall objective of this research is to determine if the soy pretzel can increase satiety relative to a conventional pretzel. The central hypothesis is that the soy pretzel will be more satiating in the short term than a traditional white, wheat pretzel. The rationale for this hypothesis relies on previous studies of other protein-rich products as well as the unique nutritional profile of soybeans (e.g., energy density, protein content) that are known to influence satiety. We propose the following aim to address this objective:

Aim: To determine the short term satiety level of soy pretzel compared to a traditional white soft pretzel. The working hypothesis is that the soy pretzel will lead to greater satiety than a conventional wheat-based pretzel.

17. RESEARCH METHODS & ACTIVITIES

a. Identify and describe all interventions and interactions that are to be performed solely for the research study. Distinguish research (i.e., experimental) activities from non-research activities. Provide data collection forms to be used. Note: Do not include case report forms for multi-site industry-sponsored or cooperative group studies.

Healthy people between the ages of 18 and 45 are able to volunteer for this study. Candidates will be exempted only if they are allergic to wheat or soy. We will advertise by posting flyers around The Ohio State University campus. If a person is interested, they will contact Amber Simmons, the study coordinator, and she will set up a meeting with them to sign the consent form and proceed with the study. 20 participants will be recruited. Each participant will be invited to the Vodovoz lab (240 Parker Food Science and Technology) and be given the consent form which, if interested, he or she will sign. The participant will be able to ask questions about the study at any time. The age and the gender of the participant will be recorded so we can accurately report the ages of our participants and so that we can calculate the percentage of males and females. To avoid bias, the participant will be told that two pretzel recipes are being tested but they will not know that one is 25% soy and one is 100% wheat. The participant will then be given a wheat pretzel and a soft pretzel (samples totaling 238 kcal; Holt et al., 1995) both in plastic ziplock bags labeled clearly with “Day 1” or “Day 2.” Whether the soy pretzel or the wheat pretzel is “Day 1” will be randomized. In addition to the pretzels, the participant will also receive written instructions on how to complete the satiety testing. Instructions will also be given orally to assure that the participant understands the procedure. The participants will be told to store the pretzels in a cool, dark place like a cabinet (not the refrigerator) until they are eaten. The participant...
will fast for 10-12 hrs overnight. Before breakfast the next morning, the participant will note his or her level of hunger on a 10-mm visual analog scale (VAS) anchored with “extremely hungry” on the left and “extremely full” on the right. He or she will then eat the pretzel marked “Day 1,” They will declare their satiety (“fullness”) then every 15 min for 2 hrs on subsequent VASes (Holt et al., 1995). Within those 2 hrs the participant should not eat any other foods or drink anything besides water. We will ask the participants to record how much water they drink within the two hours because this might affect their feeling of satiety. For the rest of the day they will be able to eat or drink whatever they want. He or she will repeat the overnight fast that night and the next morning he or she will repeat the procedure with the other pretzel. In summary, here is a time line:

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td></td>
<td>At the meeting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sign consent form</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Receive pretzel</td>
</tr>
<tr>
<td>Overnight</td>
<td></td>
<td>Fast for 10-12 hrs</td>
</tr>
<tr>
<td>Day 1</td>
<td>am</td>
<td>t=0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety (hunger/fullness), eat 1st pretzel</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>t=15 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>t=30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>45 min</td>
<td>t=45 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>t=60 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>1.5 hr</td>
<td>t=90 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>2 hrs</td>
<td>t=120 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety, record amount of water drank during the 2 hrs.</td>
</tr>
<tr>
<td>Overnight</td>
<td></td>
<td>Fast for 10-12 hrs</td>
</tr>
<tr>
<td>Day 2</td>
<td>am</td>
<td>t=0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety (hunger/fullness), eat 2nd pretzel</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>t=15 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>t=30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>45 min</td>
<td>t=45 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>t=60 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>1.5 hr</td>
<td>t=90 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety</td>
</tr>
<tr>
<td></td>
<td>2 hrs</td>
<td>t=120 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Report satiety, record amount of water drank during the 2 hrs.</td>
</tr>
</tbody>
</table>

b. Check all research activities that apply:

- Anesthesia (general or local) or sedation
- Audio, video, digital, or image recordings
- Biohazards (e.g., rDNA, infectious agents, select agents, toxins)
- Biological sampling (other than blood)
- Blood drawing
- Coordinating Center
- Data, not publicly available
- Data, publicly available
- Data repositories Complete Appendix C (future unspecified use, including research databases)
- Deception Complete Appendix D & Appendix M1
- Devices Complete Appendix E
- Diet, exercise, or sleep modifications
- Magnetic Resonance Imaging (MRI)
- Materials that may be considered sensitive, offensive, threatening, or degrading
- Non-invasive medical procedures (e.g., EKG, Doppler)
- Observation of participants (including field notes)
- Oral history (does not include medical history)
- Placebo
- Pregnancy testing
- Program Protocol (Umbrella Protocol)
- Radiation (e.g., CT or DEXA scans, X-rays, nuclear medicine procedures) Complete Appendix V
- Randomization
- Record review (which may include PHI)
- Specimen research
18. DURATION

Estimate the time required from each participant, including individual interactions, total time commitment, and long-term follow-up, if any.

The participant will come to the Vodovoz lab and be given the consent form. If the person decides to participate he or she will sign the consent form. The participant will be able to ask questions about the study at any time. After consent has been given, the participant will be given a soy pretzel and a wheat pretzel with instructions on how to do the satiety tests. This meeting will last approximately 30 min; 26 min to read and sign the consent form and 10 min to explain the satiety test. That night, the participant will fast for 10-12 hrs overnight. In the morning, the participant will declare their level of satiety and eat the first pretzel, as was assigned to them. This will take about 10 min. They will then describe how full the pretzel made them. They will declare their satiety (“fullness”) every 15 min after the first bite. Within that time the participant should not eat any other foods or drink anything besides water. For the rest of the day they will be able to eat or drink whatever they want. He or she will repeat the overnight fast that night and the next morning he or she will repeat the procedure with the other pretzel. In summary, the participant will be involved for 3 days. On day 0, the participant will pick up the pretzels for the satiety test from Vodovoz Lab. On day 1 he or she will perform one 2 hr satiety test and on day 2 he or she will perform the second 2 hr satiety test. The satiety questionnaires can be returned at their convenience but preferably as soon as possible.

19. NUMBER OF PARTICIPANTS

The number of participants is defined as the number of individuals who agree to participate (i.e., those who provide consent or whose records are accessed, etc.) even if all do not prove eligible or complete the study. The total number of research participants may be increased only with prior IRB approval.

a. Provide the total number of participants (or number of participant records, specimens, etc.) for whom you are seeking OSU IRB approval.

   20

b. Explain how this number was derived (e.g., statistical rationale, attrition rate, etc.).

   Question: Does the soy pretzel have a different satiety index than the wheat pretzel?
   The data will be analyzed with a two-sided, two-sample t-test with α=0.05 and 90% power.

   $\delta = \text{satiety(soy) - satiety(wheat)}$

   $H_0: \delta = 0$. There is no difference in satiety index between wheat and soy pretzels

   $H_1: \delta \neq 0$. There is a difference in satiety index between wheat and soy pretzels

   Define margin of error, $m = 1.5$

   Estimate standard deviation, $s = 3$

   
   $$m = t_{n-1} \left(1 - \frac{\alpha}{2}\right) \frac{s}{\sqrt{n}}$$

   $$1.5 = t_{20}(0.975) \frac{3}{\sqrt{n}}$$
We need an n of 17 to distinguish a difference in the satiety indexes of the wheat and soy pretzel if one does indeed exist. In case compliance is not 100%, we would like to recruit 20 participants.

c. Is this a multi-site study? [ ] Yes  ➔ Indicate the total number of participants to be enrolled across all sites: 
[ ] No

### 20. PARTICIPANT POPULATION

a. Specify the age(s) of the individuals who may participate in the research:
   Age(s):  

b. Specify the participant population(s) to be included (check all that apply):
   - [ ] Adults
   - [ ] Decisionally Impaired Adults  ➔ Complete Appendix M
   - [ ] Pregnant Women/Fetuses  ➔ Complete Appendix K
   - [ ] Prisoners  ➔ Complete Appendix I
   - [ ] Children (< 18 years)  ➔ Complete Appendix I
   - [ ] Healthy Volunteers
   - [ ] Neonates (uncertain viability/nonviable)  ➔ Complete Appendix K
   - [ ] Non-English Speaking  ➔ Complete Appendix I
   - [ ] Students from Participant Pools (e.g., REP)  Specify:
   - [ ] Unknown (e.g., research using secondary data/specimens, non-targeted surveys, program protocols)

c. Describe the characteristics of the population(s) and explain how the nature of the research requires/justifies inclusion of the proposed population(s).

We will be studying healthy individuals between the ages of 18 and 45 recruited from a college campus. We will be recruiting healthy people (as opposed to those with diabetes or glucose intolerance) for this a preliminary study. Moreover, this population will most likely comprise a large proportion of the soy soft pretzel consumers once the pretzels are commercialized. The only exclusion criteria are that the person cannot be allergic to wheat or soy or be pregnant.

d. Are any of the participants likely to be vulnerable to coercion or undue influence? [ ] Yes  ➔ If Yes Describe additional safeguards to protect participants’ rights and welfare.
[ ] No

e. Will pregnant women be excluded from participation in the research? [ ] Yes  ➔ If Yes Explain how the nature of the research requires/justifies their exclusion. Address means of pregnancy screening. Pregnant women will be excluded due to potential effects on glucose metabolism and requirements for fasting. A simple yes or no question will determine whether a participant is pregnant or not.
[ ] No

### 21. PARTICIPANT IDENTIFICATION, RECRUITMENT, & SELECTION

a. Provide evidence that you will be able to recruit the necessary number of participants to complete the study.
We performed an acceptability study on the same pretzel products in which 56 people were recruited easily. We performed a similar
satisfaction study last year in which 50 people were recruited. These participants were compensated with a $5 Parker Dairy Store coupon, but I predict that the absence of this incentive would not prevent their participation.

b. Describe how potential participants will be identified (e.g., advertising, individuals known to investigator, record review, etc.). Explain how investigator(s) will gain access to this population, as applicable.

Flyers will be displayed around The Ohio State University campus. The flyer will include the study coordinator’s email address so that interested people can contact her regarding the study. To respect the privacy of the participants, all satisfaction questionnaires will be labeled with the participant’s 2-digit number, not his or her name. The sheet matching each individual to their corresponding number will be discarded as soon as all raw data is obtained and will not be used for analysis or any other purpose. The study coordinator will keep all data in a locked file cabinet and all electronic data will be password protected. Raw data will not be distributed; only analyzed data will be reported and published.

c. List the names of investigator(s) and/or key personnel who will recruit participants and what process will be used to determine participant eligibility.

Amber Simmons, the study coordinator, will be in charge of recruiting individuals. Flyers will be posted around Ohio State campus stating the eligibility criteria: age 18-45, not pregnant, and not allergic to wheat or soy. Interested people can email the study coordinator. All questions will be answered via email or in person. We will simply ask the candidate if she is pregnant, if applicable, or if he or she has any wheat or soy allergies. If the person doesn’t know if he or she is allergic to soy we will discourage them from participating but not deem them ineligible.

d. Describe the recruitment process; including the setting in which recruitment will take place. Explain how the process respects potential participants’ privacy. Provide copies of proposed recruitment materials (e.g., ads, flyers, website postings, recruitment letters, and oral/written scripts).

Recruitment will begin as soon as the IRB is approved. Flyers (see attached) will be posted around the Ohio State University campus and subjects will be asked to contact the study coordinator, Amber Simmons, if interested. Amber will discuss the details of the study via email and, if the person is interested, set up a meeting for him or her to come in. At the meeting, Amber will provide the participant with a consent form. The participant will read it and ask any questions that he or she may have. After the person signs the consent form we will proceed with the study.

22. INCENTIVES TO PARTICIPATE

Will participants receive compensation or other incentives (e.g., free services, cash payments, gift certificates, parking, classroom credit, travel reimbursement) to participate in the research study? Compensation plans should be pro-rated (not contingent upon study completion) and should consider participant withdrawals, as applicable.

☐ Yes
☒ No

If Yes ☐ Describe the incentive, including the amount and timing of all payments.

23. ALTERNATIVES TO STUDY PARTICIPATION

Other than choosing not to participate, list any specific alternatives, including available procedures or treatments that may be advantageous to the subject.

There are no alternatives to participation.

24. INFORMED CONSENT PROCESS

a. Indicate the consent process(es) and document(s) to be used in the study. Check all that apply. Provide copies of documents and/or complete relevant appendices, as needed. See Consent for Research for templates or contact ORRP for more information.

☐ Assent – Form
☐ Assent – Verbal Script
☒ Informed Consent – Form
☐ Parental Permission – Form
☐ Parental Permission – Verbal Script
☐ Translated Consent/Assent – Form(s)
b. List the names of investigator(s) and/or key personnel who will obtain consent from participants or their legally authorized representatives.

Amber Simmons

The participant will provide his or her consent.

d. Describe the consent process. Explain when and where consent will be obtained and how subjects and/or their legally authorized representatives will be provided sufficient opportunity (e.g., waiting period, if any) to consider participation.

Before the meeting with the participant, the study will be explained individually to the participants via email. If a person expresses interest, he or she will be invited to the Vodovoz lab to pick up supplies. At the meeting, the first thing the subject will do is read the informed consent. At any time the participant will be allowed to ask questions. Informed consent will thus be obtained before any eating or filling out of questionnaires. The consent form will be approved by the institutional Review Board and will detail the purpose of the study, the procedures, the nature of the intervention, risks and benefits, confidentiality, and options for withdrawal from the study. Each subject will be informed both verbally and with the written consent document about the benefits and risks associated in participating in the study, and all questions concerning the study will be answered.

e. Explain how the possibility of coercion or undue influence will be minimized in the consent process.

All of the participant’s questions will be answered thoroughly and truthfully. It will be explained that each subject has the choice not to participate and will be able to leave the study at any time without stating a reason for withdrawal. There is no financial compensation or any other incentives to participate in this study.

f. Will any other tools (e.g., quizzes, visual aids, information sheets) be used during the consent process to assist participant comprehension?

☐ Yes
☐ No

Provide copies of these tools

g. Will any other consent forms be used (e.g., for clinical procedures such as MRI, surgery, etc. and/or consent forms from other institutions)?

☐ Yes
☐ No

Provide copies of these forms

25. PRIVACY OF PARTICIPANTS

a. Describe the provisions to protect the privacy interests of the participants.

Only the principle investigator and the study coordinator will have access to the questionnaires. The questionnaires will be coded and not labeled with personal data. Data that is entered into a computer will be password protected.

b. Does the research require access to personally identifiable private information?

☐ Yes
☐ No

If Yes → Describe the personally identifiable private information involved in the research. List the information source(s) (e.g., educational records, surveys, medical records, etc.).

26. CONFIDENTIALITY OF DATA

a. Explain how information is handled, including storage, security measures (as necessary), and who will have access to the information. Include both electronic and hard copy records.

Only the principle investigator and the study coordinator will have access to the questionnaires. The questionnaires will be coded and not labeled with personal data. Data that is entered into a computer will be password protected.
b. Explain if any personal or sensitive information that could be potentially damaging to participants (e.g., relating to illegal behaviors, alcohol or drug use, sexual attitudes, mental health, etc.) will be collected.  N/A

c. Will you be obtaining an NIH Certificate of Confidentiality?  Yes ⇒ Provide a copy before you begin the research  No

See OSU HRPP policy Privacy and Confidentiality for more information.

d. Explain any circumstances (ethical or legal) where it would be necessary to break confidentiality.  N/A

e. Indicate what will happen to identifiable data at the end of the study. Research-related records should be retained for a period of at least three years after the research has been discontinued (i.e., no further data collection, long term follow-up, re-contact, or analysis of identifiable/coded data.)

- Identifiers permanently removed from the data and destroyed (de-identified)
- Identifiable/coded (linked) data are retained
- Identifiable data not collected

27. HIPAA RESEARCH AUTHORIZATION

Will individually identifiable Protected Health Information (PHI) subject to the HIPAA Privacy Rule requirements be accessed, used, or disclosed in the research study?

- No
- Yes ⇒ Check all that apply:
  - Written Authorization ⇒ Provide a copy of the Authorization Form
  - Partial Waiver (recruitment purposes only) ⇒ Complete Appendix N
  - Full Waiver (entire research study) ⇒ Complete Appendix N
  - Alteration (written documentation) ⇒ Complete Appendix N

28. REASONABLY ANTICIPATED BENEFITS

a. List the potential benefits that participants may expect as a result of this research study. State if there are no direct benefits to individual participants. Compensation is not to be considered a benefit.

There is no direct benefit to the participants of the study.

b. List the potential benefits that society and/or others may expect as a result of this research study.

Society will gain knowledge of the satiety index of soy-based baked goods compared to wheat-based baked goods. This is especially important for the diabetic community and anyone who is trying to lose weight by eating less. Trends towards more filling snack foods may help prevent obesity in the developed world.

29. RISKS, HARMs, & DISCOMFORTS

a. Describe all reasonably expected risks, harms, and/or discomforts that may apply to the research. Consider the range of risks, including physical, psychological, social, legal, and economic. As applicable, discuss severity and likelihood of occurrence. It is possible, although rare, for a participant to have never encountered soy before and be allergic. If an allergic reaction occurs during the satiety test the person will be encouraged to seek help immediately and not complete the satiety survey. About 1% of the population has soy allergies and most are diagnosed when the person is still an infant or a small child. In most cases, allergic reactions to soy are mild and include redness, rash, inflammation, or hives but can be as severe as anaphylactic shock.

b. Describe how risks, harms, and/or discomforts will be minimized.
The person will be asked directly if they have had soy products before (soy sauce counts) to assure that they are not allergic to soy. This will occur in the preliminary emails, before the meeting is set up. If they do not know, the study coordinator will discourage participation.

30. MONITORING

Does the research involve greater than minimal risk (i.e., are the harms or discomforts described in Question #29 beyond what is ordinarily encountered in daily life or during the performance of routine physical or psychological tests)?

- Yes
- No

If Yes  
Describe the plan to oversee and monitor data collected to ensure participant safety and data integrity. Include the following:

- The information that will be evaluated (e.g., incidence and severity of actual harm compared to that expected);
- Who will perform the monitoring (e.g., investigator, sponsor, or independent monitoring committee);
- Timing of monitoring (e.g., at specific points in time, after a specific number of participants have been enrolled); and
- Decisions to be made as a result of the monitoring process (e.g., provisions to stop the study early for unanticipated problems).

31. ASSESSMENT OF RISKS & BENEFITS

Discuss how risks to participants are reasonable when compared to the anticipated benefits to participants (if any) and the importance of the knowledge that may reasonably be expected to result.

Risk for the participants will be minimal. Some participants may experience some flatulence if soy is not a common food in their diet. However, the risk of pain is small. We will tell each participant to immediately discontinue the study if they notice any signs of allergic reactions. Knowledge gained from this study will supplement the currently limited knowledge pool on soy products. It could potentially change how dieters view soy-enhanced products and could potentially influence the direction of food companies that heed the satiety index.

32. PARTICIPANT COSTS/REIMBURSEMENTS

a. List any potential costs subjects (or their insurers) will incur as a result of study participation (e.g., parking, study drugs, diagnostic tests, etc.).

The participant may need to pay for parking. Most participants, however, are expected to be students and can walk or take the CABS bus from campus.

b. List any costs to participants that will be covered by the research study.

The cost to produce and distribute the pretzels, the time and effort of the study coordinators, and all future data analyses will be covered by the research study.
33. APPLICATION CONTENTS

Indicate the documents being submitted for this research project. Check all appropriate boxes.

☑ Initial Review of Human Subjects Research Application
☑ Appendix A1: OSU Co-Investigators & Key Personnel (questions 4 & 5)
☑ Appendix A2: External (non-OSU) Co-Investigators & Key Personnel (question 6)
☑ Appendix B: Expedited Review – Initial Review (question 13)
☑ Appendix C: Data Repositories (question 17b)
☑ Appendix D: Deception (question 17b)
☑ Appendix E: Devices (question 17b)
☑ Appendix F: Drugs or Biologics (question 17b)
☑ Appendix G: Genetic Testing (question 17b)
☑ Appendix H: Storage of Biological Materials (question 17b)
☑ Appendix I: Children (question 20b)
☑ Appendix J: Non-English Speaking Participants (questions 20b and 24a)
☑ Appendix K: Pregnant Women/Fetuses/Neonates (question 20b)
☑ Appendix L: Prisoners (question 20b)
☑ Appendix M1: Waiver or Alteration of Consent Process (questions 17b & 24a)
☑ Appendix M2: Waiver of Consent Documentation (question 24a)
☑ Appendix N: Waiver of HIPAA Research Authorization (question 27)
☑ Appendix U: Research in International Settings (question 12)
☑ Appendix V: Radiation (question 17b)
☑ Appendix W: Decisionally Impaired Adults (question 20b)
☑ Consent form(s), Assent Form(s), Permission Form(s), and Verbal Script(s), including translated documents (question 24a)
☑ HIPAA Research Authorization Form(s) (question 27)
☑ Data Collection Form(s) (question 17a)
☑ Data Collection Forms involving protected health information (Appendix N)
☑ Recruitment Materials (e.g., ads, flyers, telephone or other oral script, radio/TV scripts, internet solicitations) (question 21d)
☑ Script(s) or Information Sheet(s), including Debriefing Materials (question 24)
☑ Instruments (e.g., questionnaires or surveys to be completed by participants) (question 17b)
☑ Other Committee Approvals/Letters of Support (questions 11 & 12)
☑ Research Protocol
☑ Complete Grant Application or Funding Proposal
☑ Drug Manufacturer’s Approved Labeling/Investigator’s Drug Brochure (Appendix F)
☑ Device Manufacturer’s Approved Labeling (Appendix E)
☑ Other supporting documentation and/or materials

For Multi-Site Clinical Trials supported by DHHS, the submission will also include:

☑ DHHS-approved Sample Informed Consent Document (if one exists)
☑ DHHS-approved Protocol (if one exists)
34. ASSURANCE

PRINCIPAL INVESTIGATOR (or Advisor)

I agree to follow all applicable policies and procedures of The Ohio State University and federal, state, and local laws and guidance regarding the protection of human subjects in research, as well as professional practice standards and generally accepted good research practice guidelines for investigators, including, but not limited to, the following:

- Perform the research as approved by the IRB under the direction of the Principal Investigator (or Advisor) by appropriately trained and qualified personnel with adequate resources;
- Initiate the research after written notification of IRB approval has been received;
- Obtain and document (unless waived) informed consent and HIPAA research authorization from human subjects (or their legally authorized representatives) prior to their involvement in the research using the currently IRB-approved consent form(s) and process;
- Promptly report to the IRB events that may represent unanticipated problems involving risks to subjects or others;
- Provide significant new findings that may relate to the subjects willingness to continue to participate;
- Inform the IRB of any proposed changes in the research or informed consent process before changes are implemented, and agree that no changes will be made until approved by the OSU IRB (except where necessary to eliminate apparent immediate hazards to participants);
- Complete and submit a Continuing Review of Human Subjects Research application before the deadline for review at intervals determined by the IRB to be appropriate to the degree of risk (but not less than once per year) to avoid expiration of IRB approval and cessation of all research activities;
- Maintain research-related records (and source documents) in a manner that documents the validity of the research and integrity of the data collected, while protecting the confidentiality of the data and privacy of participants;
- Retain research-related records for audit for a period of at least three years after the research has ended (or longer, according to sponsor or publication requirements) even if I leave the University;
- Contact the Office of Responsible Research Practices for assistance in amending (to request a change in Principal Investigator) or terminating the research if I leave the University or am unavailable to conduct or supervise the research personally (e.g., sabbatical or extended leave);
- Provide a Final Study Report to the IRB when all research activities have ended (including data analysis with individually identifiable or coded private information); and
- Inform all Co-Investigators, research staff, employees, and students assisting in the conduct of the research of their obligations in meeting the above commitments.

I verify that the information provided in this Initial Review of Human Subjects Research application is accurate and complete.

Signature of Principal Investigator (or Advisor) ____________________________ Date __________

Printed name of Principal Investigator (or Advisor)

______________________________

DEPARTMENT CHAIR (or Signatory Official)

As Department Chair (or Signatory Official) for the Principal Investigator, I acknowledge that this research is in keeping with the standards set by our unit and that it has met all Departmental/College requirements for review.

If the PI or any Co-Investigator is also the Department Chair, the signature of the Dean or other appropriate Signatory Official, such as the Associate Dean for Research, must be obtained.

Signature of Department Chair ____________________________ Date __________

Printed name of Department Chair

______________________________
APPENDIX A1
OSU Co-Investigators & Key Personnel

Complete this form to list OSU Co-Investigators and key personnel on the research study. Signatures are required of all OSU Co-Investigators. Use Appendix A2 to list external (non-OSU) Co-Investigators and key personnel.

Key personnel are defined as individuals who participate in the design, conduct, or reporting of human subjects research. At a minimum, include individuals who recruit or consent participants or who collect study data.

All OSU individuals listed on this protocol will have access to information about IRB actions and the completion status of each individual’s administrative and training requirements (CITI, COI disclosure). Note: Personal financial information provided in COI disclosures is not included.

PI Name: Yael Vodovotz

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<tr>
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<th>Miller, Carla K.</th>
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<td>University Academic Title:</td>
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<td>Education and Human Ecology</td>
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<td><a href="mailto:cmiller@ehe.osu.edu">cmiller@ehe.osu.edu</a></td>
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Signature of Co-Investigator

Carla Miller

Printed name of Co-Investigator

Name (Last, First, MI): 
University Academic Title:
Department Name (TIU):
Department # (TIU):
OSU ID Number:

Signature of Co-Investigator

Date

Printed name of Co-Investigator

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University Academic Title:
Department Name (TIU):
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Signature of Co-Investigator

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Appendix F: Recruitment Flyer for Satiety Study
Soft Pretzel Satiety Study

**WHO:**
Anyone age 18-45 who is not allergic to wheat or soy and not pregnant.

**WHAT:**
We are determining the differences in satiety between two different soft pretzel varieties. We would like you to eat two varieties of pretzel for breakfast on two consecutive days and tell us how full they make you over the course of the next two hours.

**WHY:**
Foods that have a high satiety index will fill you up more and result in less food consumed total. These foods can help prevent and treat obesity. We are developing a more healthful soft pretzel and are investigating whether either of these pretzel varieties is more filling than the other.

**RISKS AND BENEFITS:**
The risk in this study is extremely minor unless you are allergic to wheat or soy.

**HOW:**
If you or anyone you know might be interested, please contact Amber Simmons at simmons.225@osu.edu for more information.
Appendix G: Consent Form for Satiety Study
The Ohio State University Consent to Participate in Research

Study Title: Determination of the Satiety of Two Soft Pretzels

Researcher: Yael Vodovotz, Ph.D.

Sponsor: Ohio Agricultural Research and Development Center

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate.

Your participation is voluntary.

Please consider the information carefully. Feel free to ask questions before making your decision whether or not to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form.

Purpose: Foods that have a high satiety index will fill you up more and result in less food consumed total. These foods can help prevent and treat obesity. We are developing a more healthful soft pretzel and are investigating whether either of these pretzel varieties is more filling than the other.

Procedures/Tasks: You will eat one pretzel from two different recipes for breakfast on two consecutive days. Before eating the pretzel you will fill out a questionnaire describing how hungry you are. Then, you will report how full the pretzel made you 15 min after you take the first bite. You will report your satiety (fullness) again 30 min, 45 min, 1 hr, 1.5 hrs, and 2 hrs after you eat the pretzel. You may consume nothing besides water for these two hours.

Duration: The study will last 3 days, including today.

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

Risks and Benefits: The risks of this study are extremely low unless you are allergic to wheat or soy. If you have not had soy before you are discouraged from participating but not disallowed. There are no direct benefits for participation.
Confidentiality:

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law. Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor, if any, or agency (including the Food and Drug Administration for FDA-regulated research) supporting the study.

Incentives: None

Participant Rights:

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

Contacts and Questions:

For questions, concerns, or complaints about the study you may contact Amber Simmons, the study coordinator, at simmons.225@osu.edu or 614-247-7686.

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Yael Vodovotz at vodovotz.1@osu.edu.
Signing the consent form

I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

I am not giving up any legal rights by signing this form. I will be given a copy of this form.

Printed name of subject

Signature of subject

Date and time

Printed name of person authorized to consent for subject (when applicable)

Signature of person authorized to consent for subject (when applicable)

Relationship to the subject

Date and time

Investigator/Research Staff

I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent

Signature of person obtaining consent

Date and time
Appendix H: Instructions Given to the Participants for the Satiety Study

Soft Pretzel Satiety Study
Instructions

Store your pretzels in the clear plastic bag at room temperature until you eat them. On day 1, eat the pretzel marked “Day 1” and fill out the sheet marked “Day 1”. On day 2, use the pretzel and the corresponding questionnaire for day 2.

You will be eating these pretzels for breakfast on two consecutive days. Make sure that you fast overnight for at least 10-12 hours before you eat the pretzel. When it is time for breakfast, first mark on the scale how hungry you are with a vertical line (example below).

[Scale image]

Extremely hungry                          Extremely full

Then, eat the pretzel as is. Do not toast, microwave, add salt, or prepare the pretzel for eating in any way. Do not eat or drink anything besides water with the pretzel or for the next 2 hours. This includes coffee and tea! (Sorry) Write down precisely when you take the first bite of the pretzel and try to eat the pretzels within 10 min. Exactly 15 min after you take the first bite, mark how full the pretzel made you. Repeat this procedure 30 min, 45 min, 1 hr., 1.5 hrs, and 2 hrs after you took the first bite of the pretzel. Also, please estimate how much water you drink within the 2 hour time period and write that in the indicated space. Feel free to make any comments about the pretzel, your state of satiety, or any other relevant topics at the bottom or on the back of the paper. After two hours feel free to eat or drink anything you like.

After the completion of the questionnaire for day 2 please return the papers to Amber Simmons in 240 Parker FST. Please return them within one week of your completion of the second pretzel.

If you have any questions at all please contact Amber, the study coordinator, at simmons.225@osu.edu.
Appendix I: Questionnaire for Satiety Study
Soft Pretzel Satiety Questionnaire

Immediately before the first bite: Time:____________

How hungry/full do you feel?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Extremely hungry</td>
<td>Extremely full</td>
</tr>
</tbody>
</table>

Time of the first bite: _______________

15 minutes after the first bite: Time:____________

How hungry/full do you feel?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Extremely hungry</td>
<td>Extremely full</td>
</tr>
</tbody>
</table>

30 minutes after the first bite: Time:____________

How hungry/full do you feel?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely hungry</td>
<td>Extremely full</td>
</tr>
</tbody>
</table>

45 minutes after the first bite: Time:____________

How hungry/full do you feel?

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely hungry</td>
<td>Extremely full</td>
</tr>
</tbody>
</table>

(over →)
Day: __________

60 minutes (1 hour) after the first bite: Time: __________

How hungry/full do you feel?

_____________________________________________________________________
Extremely hungry                      Extremely full

90 minutes (1.5 hours) after the first bite: Time: __________

How hungry/full do you feel?

_____________________________________________________________________
Extremely hungry                      Extremely full

2 hours after the first bite: Time: __________

How hungry/full do you feel?

_____________________________________________________________________
Extremely hungry                      Extremely full

How much water did you drink over the course of the two hours? ________________

Please write any comments below. Thanks for your participation!
Appendix J: Ohio State University Institutional Review Board

Satiety Protocol: Exempt Approval
July 28, 2010

Protocol Number: 2010E0504
Protocol Title: DETERMINATION OF THE SATIETY OF TWO SOFT PRETZELS, PART II, YAEI VODOVOTZ, CARLA MILLER, FOOD SCIENCE & TECHNOLOGY
Type of Review: Request for Exempt Determination

Dear Dr. Vodovotz,

The Office of Responsible Research Practices has determined the above referenced protocol exempt from IRB review.

Date of Exempt Determination: 7/27/2010
Qualifying Exemption Category: 6

Please note the following:

- Only OSU employees and students who have completed CITI training and are named on the signature page of the application are approved as OSU Investigators in conducting this study.
- No changes may be made in exempt research (e.g., personnel, recruitment procedures, advertisements, instruments, etc.). If changes are need, a new application must be submitted.
- Per university requirements, all research-related records (including signed consent forms) must be retained and available for audit for a period of at least three years after the research has ended.
- It is the responsibility of the Investigator to promptly report events that may represent unanticipated problems involving risks to subjects or others.

This determination is issued under The Ohio State University’s OHRP Federalwide Assurance #00006378. All forms and procedures can be found on the ORRP website – www.orrp.osu.edu. Please feel free to contact the ORRP staff contact listed below with any questions or concerns.

Cheri Pettey, MA, Certified IRB Professional
Senior Protocol Analyst—Exempt Research
Office of Responsible Research Practices
Ohio State University
1960 Kenny Road
Columbus, OH 43210
phone: 614.688.0389
fax: 614.688.0366
email: pettey.6@osu.edu

Exempt Determination
Version 1.2
INITIAL REVIEW OF HUMAN SUBJECTS RESEARCH
The Ohio State University Institutional Review Boards

Office of Responsible Research Practices (ORRP)
300 Research Foundation Building, 1960 Kenny Road, Columbus, OH 43210
Phone: (614) 688-8457  Fax: (614) 688-0366  www.orrp.osu.edu

<table>
<thead>
<tr>
<th>ORIG. DATE</th>
<th>DATE RECEIVED:</th>
<th>DATE VERIFIED COMPLETE:</th>
<th>OSU PROTOCOL NUMBER:</th>
</tr>
</thead>
</table>

1. PROJECT TITLE:
Determination of the glycemic and insulin indexes of a soy pretzel in healthy individuals.

2. INSTITUTIONAL REVIEW BOARD
Select the Board to review this research:  □ Behavioral and Social Sciences
                                             ☑ Biomedical Sciences
                                             □ Cancer

Final Board assignment is determined by ORRP.

3. PRINCIPAL INVESTIGATOR (or Advisor) - see Qualifications for service as a PI
Name (Last, First, M.I.): Vodovotz, Yael  Degree(s): PhD
University Academic Title: Associate Professor  College (TIU): Food, Agricultural, and Environmental Sciences
Department Name (TIU): Food Science and Technology  Department #: (TIU): 1156
Campus Mailing Address: 2015 Fyffe Ct.  OSU ID Number: 00093653
Columbus, OH 43210
E-mail: vodovotz.1@osu.edu  Fax: 614-292-0218
Phone: 614-247-7696  Emergency phone: 614-578-4303

4. CO-INVESTIGATOR(S)
Are there any OSU Co-Investigators on this protocol? ☑ Yes  Complete Appendix A1
                                               □ No

5. KEY PERSONNEL
Are there any OSU key personnel on this protocol? ☑ Yes  Complete Appendix A1
                                               □ No

6. EXTERNAL CO-INVESTIGATOR(S) & KEY PERSONNEL
Are any external (non-OSU) Investigators or key personnel engaged in the OSU research?  □ Yes
                                      ☑ No  Go to Question #7

If Yes  Who will provide approval for these external personnel?  ☑ OSU IRB  Complete Appendix A2
                                               □ Non-OSU IRB  Provide a copy of the approval(s)
7. ADDITIONAL CONTACT(S)  □ N/A
If further information about this application is needed, specify the contact person(s) if other than the PI (e.g., study or regulatory coordinator, research assistant, etc.).

Name (Last, First, MI):  Amber Simmons  
Phone:  614-247-7686
E-mail:  simmons.225@osu.edu  
Fax:  614-292-0218

Name (Last, First, MI):  
Phone:
E-mail:  
Fax:

8. EDUCATION
Have all OSU investigators and key personnel completed the required web-based course (CITI) in the protection of human research subjects?  □ Yes  □ No

9. CONFLICT OF INTEREST
Does any OSU investigator (including principal or co-investigator), key personnel, or their immediate family members have a significant financial interest (e.g., speaking and consultation fees, travel expenses, proprietary interest in the tested product, stock ownership or other equity or membership in the sponsor over $10,000 per year or representing greater than 5% ownership in the sponsor) with the entity supporting the research or any company that may benefit from the research?  □ Yes  □ No

10. FUNDING OR OTHER SUPPORT
a. Is the research funded or has funding been requested?  □ Yes  □ No

If Yes → Specify sponsor: OARDC Seeds grant and provide OSU RF project number:

b. Is there any support other than monetary (e.g., drugs, equipment, etc.) being provided for the study?  □ Yes  □ No

If Yes → Specify: Soy-based and wheat pretzel and blood glucose testing

11. OTHER INSTITUTIONAL APPROVALS
Check all that apply and provide applicable documentation. See websites listed below for information on obtaining approvals.

□ None

□ Clinical Research Center (CRC) Scientific Advisory Committee (SAC) – Approval required for research sponsored by the CRC. Final IRB approval will be held pending receipt of SAC approval. See www.gerc.osu.edu.

□ Institutional Biosafety Committee (IBC) – Approval required for research involving bioshazards (recombinant DNA, infectious or select agents, toxins), gene transfer, or xenotransplantation. See http://orrb.osu.edu/ibc or contact ORRP.

□ Comprehensive Cancer Center (CCC) Clinical Scientific Review Committee (CSRC) – Approval or exemption required for cancer-related research. See www.osuccc.osu.edu/csre or contact the CCC Clinical Trials Office.

□ Maternal-Fetal Welfare Committee – Approval required for some research involving pregnant women and fetuses. See http://orrb.osu.edu/irb/osupolicies/MFWreview.cfm or contact ORRP.

□ Human Subjects Radiation Committee (HSRC) – Approval required for research involving radiological procedures for research purposes (e.g., non-clinical care X-rays, DEXA or CT scans, nuclear medicine procedures, etc.). See www.ehs.ohio-state.edu or contact ORRP.
12. LOCATION OF THE RESEARCH

a. List the specific site(s) at which the OSU research will be conducted (include both domestic and international locations).

<table>
<thead>
<tr>
<th>Location Name (or description)</th>
<th>Address (street, city and state, or country)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Clinical Research Center</td>
<td>Davis Medical Research Center, 480 Medical Center Dr., Columbus OH</td>
</tr>
<tr>
<td>Vodovotz Lab</td>
<td>240 Parker Food Science and Technology, 2015 Fyffe Ct., Columbus OH</td>
</tr>
</tbody>
</table>

b. Are all the sites named above on the OSU list of approved research performance sites? See http://orp.osu.edu/ehs/units/cies/research-sites.cfm.

☐ Yes ☐ No

If No ⇒ ☐ Domestic sites ⇒ Provide a letter of support, as applicable
☐ International sites ⇒ Complete Appendix I

13. EXPEDITED REVIEW

Are you requesting Expedited Review?

☐ Yes ⇒ Complete Appendix B
☐ No

14. SUMMARY OF THE RESEARCH

Summarize the proposed research using non-technical language that can be readily understood by someone outside the discipline. Explain briefly the research design, procedures to be used, risks and anticipated benefits, and the importance of the knowledge that may reasonably be expected to result. Use complete sentences (limit 300 words).

The bulk of research regarding the nutritional benefits of soy foods has focused on the protection against cancer and heart disease, mainly via soy isoflavones and soy protein, respectively. However, due to the unique composition of soy foods they may beneficially impact postprandial glycemia and satiety, which may in turn help to reduce the risk of obesity and diabetes. A highly acceptable soy soft pretzel containing 8 g protein per serving has been developed at OSU and is being commercialized. The objective of this proposal is to determine the blood glucose and insulin responses of this soy pretzel and compare these values to the effects of conventional soft pretzels in healthy adults. Preliminary results from this study would lay the foundation for larger studies to examine the effects of daily soy intake on overall glycemic control in patients with diabetes and on long-term regulation of food intake for weight management.

15. SCIENTIFIC BACKGROUND & LITERATURE REVIEW

Summarize existing knowledge and previous work that support the expectation of obtaining useful results without undue risk to human subjects. Use complete sentences (limit 300 words).

Several studies have documented that a soy bread made with a patent pending technology and formulation developed at The Ohio State University had excellent consumer acceptability, improved shelf-life, and that its isoflavone content was bioavailable. However, in a short-term test market in the Columbus area, consumers expressed a desire for a snack like item with the same qualities. Therefore, a soy soft pretzel has been developed at a Cincinnati bakery using this technology and formulation. Preliminary results showed a highly acceptable product delivering 8 grams of soy protein per serving.

A review by Fiona Atkinson, et al. compiled glycemic index and glycemic load results of all literature in MEDLINE between January 1981 and December 2007. Three soy/fiberbread (flax seed) breads were reported with glycemic indexes of 36 ± 4, 50 ± 6, and 55 ± 4 with glucose as a reference. The average glycemic index for white wheat bread is 75 ± 2 and that for whole wheat bread is 74 ± 2, according to the same report. No values for soft pretzels were reported. The effects of any soy enhanced bakery formulation in the absence of linseed on postprandial glycemia (i.e., GI) have not been determined. The proposed project will provide such information as well as insight into future improvements that could be made to the soft pretzel to help people achieve better blood glucose control and maintenance of
healthy body weight.

16. RESEARCH OBJECTIVES

List the specific scientific or scholarly aims of the research study.

Our long-term goal is to develop new dietary strategies for the maintenance of healthy blood glucose levels, especially with regard to diabetes and pre-diabetes. The overall objective of this research is to determine how the consumption of a soy-enhanced soft pretzel affects the postprandial glucose and insulin responses. The central hypothesis is that the soy pretzel will have a lower glucose and insulin response relative to conventional pretzel. The rationale for this hypothesis relies on previous studies of other soy products and their effect on glycemia. We propose the following objective to address this objective:

To determine the glucose and insulin responses of soft soy pretzels in healthy subjects. The working hypothesis is that the soy pretzel will have a lower glucose and insulin response (as defined by a GI <55 relative to a glucose standard) than a conventional pretzel. Further, the soy pretzel will have a reduced glycemic index as compared with the conventional pretzel when both foods are referenced against a glucose standard.

17. RESEARCH METHODS & ACTIVITIES

a. Identify and describe all interventions and interactions that are to be performed solely for the research study. Distinguish research (i.e., experimental) activities from non-research activities.

Subjects will be screened initially for fasting glucose level (Accucheck Advantage Blood Glucose Monitoring System, Roche Diagnostics, Indianapolis, IN) and will fill out a health questionnaire for information on medical conditions and use of medications or dietary supplements that could affect glucose tolerance or food intake. Anthropometric measures (height, weight) will also be obtained.

The protocol for GI testing will be standardized via the methods published by Wolke et al. The GI will be determined on a soy soft pretzel and wheat soft pretzel as well as glucose and white bread as controls. Eligible subjects will taste each of the pretzels and white bread in random order. In addition, subjects will be required to complete a total of 3 replications with the glucose standard as recommended for GI standardization. The white bread will serve as a validation of methods; we expect a GI of 75 ± 2 as reported in the International Tables of Glycemic Index and Glycemic Load Values: 2008 (Atkinson, et. al). Therefore, the subjects will complete a total of 6 treatment visits (1 trial for the wheat pretzel, 1 trial for the soy pretzel, 1 trial for the white bread, and 3 trials with the glucose standard). For 3 days prior to each treatment visit, subjects will complete dietary record forms to insure that a carbohydrate intake of at least 159 g/d was consumed and normal glycogen stores are present. On the night before the treatment visit, subjects will begin fasting after the evening meal (an overnight fast of 10 – 12 h). Upon the arrival of the subject to the laboratory the next morning (typically about 0700 h), the subject’s temperature and blood pressure will be recorded and the subject will rest quietly for 30 min. If dietary records indicate inadequate carbohydrate intake or if temperature or blood pressure are not within normal range, the treatment visit will be re-scheduled. During the 30 min rest period, intravenous soft tube (catheter) will be placed in the upper arm in a vein to obtain blood samples. At the completion of the 30 min rest period, baseline blood glucose and insulin concentrations will be measured. Immediately thereafter, the subject will be fed a meal consisting of a portion size of the test food that is calculated from the food label to provide 50 g available carbohydrate (total carbohydrate minus dietary fiber) and 240-300 mL of tap water (volume similar for each test). The glucose standard will consist of an orange-flavored glucose tolerance test beverage that contains 50 g anhydrous glucose in 30 mL of water (Fisherbrand, Fisher Health Care, Houston, TX). Timing of subsequent blood sampling will start with the first bite of the test meal. The subject will resume fasting (sips of water only) after the test meal and blood samples will be drawn to determine glucose and insulin concentrations at 15, 30, 45, 60, 90, and 120 min after the start of the test meal. Only minimal physical activity will be allowed during the blood testing. A minimum of 96 h will separate each treatment visit. 6 mL of serum will be collected for the insulin response assay and 5 mL of serum will be collected for the glucose assay. Total blood collected will be: [(6 mL/insulin assay + 5 mL/glucose assay)] * (7 time points/day) * (6 days) = 462 mL blood over the course of the entire experiment.

It is recommended that the glucose standard (gic std.) be administered as the first sample, the last sample, and one in the middle in order to best gauge the standard. The participants will be randomly assigned to one of three groups which will sample the pretzels and the bread in different orders. The schedule is as followed:
b. Check all research activities that apply:

- Anesthesia (general or local) or sedation
- Audio, video, digital, or image recordings
- Biohazards (e.g., rDNA, infectious agents, select agents, toxins)
- Biological sampling (other than blood)
- Blood drawing
- Coordinating Center
- Data, not publicly available
- Data, publicly available
- Data repositories → Complete Appendix C (future unspecified use, including research databases)
- Deception → Complete Appendix D & Appendix M1
- Devices → Complete Appendix E
- Diet, exercise, or sleep modifications
- Drugs or biologics → Complete Appendix F
- Emergency research
- Focus groups
- Food supplements
- Gene transfer
- Genetic testing → Complete Appendix G
- Internet or e-mail data collection
- Magnetic Resonance Imaging (MRI)
- Materials that may be considered sensitive, offensive, threatening, or degrading
- Non-invasive medical procedures (e.g., EKG, Doppler)
- Observation of participants (including field notes)
- Oral history (does not include medical history)
- Placebo
- Pregnancy testing
- Program Protocol (Umbrella Protocol)
- Radiotopes or other sources of ionizing radiation
- Radioactive materials (requires approval from Radiation Safety Committee)
- Randomization
- Record review (which may include PHI)
- Specimen research
- Stem cell research
- Storage of biological materials → Complete Appendix H (future unspecified use, including repositories)
- Surgical procedures (including biopsies)
- Surveys, questionnaires, or interviews (one-on-one)
- Surveys, questionnaires, or interviews (group)
- X-rays or microwaves
- Other
- Specify:

18. DURATION

Estimate the time required from each participant, including long-term follow-up, if any. Describe the time commitment in detail.

Initial screening will last about one hour. During initial screening we will collect anthropometric data (height, weight), ask about the chances of pregnancy, and inquire about tobacco use. We will thoroughly review the consent form with the subject and, if he or she is interested, the subjects will give consent. For all subsequent visits, the subject will arrive at the laboratory in the morning after an overnight fast (10 – 12 hrs). We will measure the subject’s temperature and blood pressure and the subject will provide a dietary record of their food intake for the past 3 days. The subject will then rest quietly for 30 min and have the catheter put into place. At the completion of the 30 min rest period, a blood sample will be obtained for the baseline glucose and insulin levels. Immediately thereafter, the subject will consume a pretzel, slice of bread, or glucose standard. At 15, 30, 45, 60, 90, and 120 min after the first bite, blood samples for glucose and insulin will be obtained. Therefore, each visit should have a duration of approximately 2 hrs, 45 min. (15 min
for measurement of temperature and blood pressure (+ 30 min rest period + 120 min blood sample collection). Participants will need to make 6 visits to the laboratory with a minimum of 96 hours in between visits.

19. NUMBER OF PARTICIPANTS

a. Provide the maximum number of participants (or number of participant records, specimens, etc.) for whom you are seeking OSU IRB approval. 12

b. Explain how this number was derived.

In a detailed review of glycemic index methodology, Wolever, et al. discussed how many subjects should be enrolled for glycemic index testing. They performed a simulation analysis to investigate the effect of sample size on the detection of differences in the glycemic index (GI) between two foods at a level of p<0.05 with 80% power. With an estimate of the GI of 60, the model predicts a detectable difference of 16 with 10 subjects. Based on previous studies, we hypothesize the difference in GI between the wheat and soy pretzel to be much larger than 16, perhaps even 20 to 25. We will recruit 2 more subjects than 10 in case some decide to discontinue the study.

c. Is this a multi-center study? ☐ Yes ☐ No

The total number of research participants may be increased only with prior IRB approval.

20. PARTICIPANT POPULATION

a. Specify the age(s) of the individuals who may participate in the research:

Age(s): 18-45

b. Specify the participant population(s) to be included (check all that apply):

☐ Adults
☐ Adults unable to consent for themselves
☐ Children (< 18 years) → Complete Appendix 1
☐ Healthy volunteers
☐ Neonates (uncertain viability/nonviable) → Complete Appendix K
☐ Pregnant Women/Fetuses → Complete Appendix K
☐ Prisoners → Complete Appendix 1
☐ Students from participant pools (e.g., REP)
☐ Unknown (e.g., research using secondary data/specimens, non-targeted surveys, program protocols)

Specify:

Non-English speaking → Complete Appendix J

We will be studying healthy individuals between the ages of 18 and 45 recruited from a college campus. We will be recruiting healthy people (as opposed to those with diabetes or glucose intolerance) for this a preliminary study. Moreover, this population will most likely comprise a large proportion of the soy soft pretzel consumers once the pretzels are commercialized.

d. Will pregnant women be excluded from participation in the research? ☐ Yes ☐ No

If Yes, explain how the nature of the research requires/justifies their exclusion. Address means of pregnancy screening.

Pregnant women will be excluded due to potential effects on glucose metabolism and requirements for fasting. A simple yes or no question will determine whether a participant is pregnant or not.

21. PARTICIPANT IDENTIFICATION, RECRUITMENT, & SELECTION

a. Describe how potential participants will be identified (e.g., advertising, individuals known to investigator, record review, etc.).

Explain how the method(s) for identifying potential participants respects their privacy.
Flyers will be displayed around The Ohio State University campus. To respect the privacy of the participants, all data and samples will be labeled with a double-digit number, not their name (01-12). The GCRC will add their own number to all blood samples, but each of these numbers will be superseded by their double-digit study number. The sheet matching each individual to their corresponding number will be discarded as soon as all raw data is obtained and will not be used for analysis or any other purpose. The study coordinator will keep all data in a locked file cabinet and all electronic data will be password protected. Raw data will not be distributed; only analyzed data will be reported and published.

b. State who (investigators and/or key personnel) will recruit participants and what process will be used to determine participant eligibility.

The study coordinator, Amber Simmons, will be in charge of recruiting participants. The flyer will advertise the study to healthy nonsmokers without history of diabetes, glucose intolerance, or gastrointestinal disorders and will include Amber’s email address. When she is contacted, she will give them information about the study and thoroughly answer any questions via email. The study coordinator will then schedule the initial screening for interested individuals. Candidates will be invited to come for an initial screening and (after signing an informed consent) will fill out a health questionnaire and provide anthropometric measurements. Participants will be excluded if they (1) have medical conditions that could affect glucose tolerance or food intake, (2) use medications or dietary supplements that could affect glucose tolerance or food intake, (3) smoke, (4) are pregnant or are lactating, or (5) have a body mass index (BMI) of 30 kg/m² or greater. The study coordinator will work in conjunction with Drs. Yael Vedovotz and Carla Miller to determine eligibility.

c. Describe the recruitment process; including how and where recruitment will take place. Provide copies of proposed recruitment materials (e.g., ads, flyers, website postings, recruitment letters, and oral/written scripts).

Recruitment will begin as soon as the IRB is approved. Flyers (see attached) will be posted around the Ohio State University campus and subjects will be asked to contact the study coordinator, Amber Simmons, if interested. Amber will discuss the details of the study via email and, if interested, the subject will be invited to come for the initial screening. Scheduling will occur on a first-come-first-serve basis. The first manner of business at the initial screening will be for the subject to sign the informed consent. If the subject agrees, the study coordinator can send the consent form via email and the subject can read it before they arrive. Either way, the subject will read the consent form and have any questions answered before they sign it. Then, the health questionnaire and anthropometric measurements will be obtained and eligibility will be determined. Those that are eligible and interested will be enrolled for the study.

22. INCENTIVES TO PARTICIPATE

Will participants receive compensation or other incentives (e.g., free services, cash payments, gift certificates, parking, classroom credit, travel reimbursement) to participate in the research study? □ Yes □ No

If Yes → Describe the incentive. Compensation should be pro-rated (e.g., per visit) and not contingent upon study completion. Compensation will be $300 per subject ($50 per visit).

23. INFORMED CONSENT PROCESS

a. Indicate the consent process(es) and document(s) to be used in the study. Check all that apply.

☐ Asent – Form ☐ Parental Permission – Form
☐ Asent – Verbal Script ☐ Parental Permission – Verbal Script → Complete Appendix M2
☒ Informed Consent – Form ☐ Translated Consent/Asent – Form(s) → Complete Appendix J
☐ Informed Consent – Verbal Script → Complete Appendix M2
☐ Waiver or Alteration of Consent Process → Complete Appendix M1
☐ Informed Consent – Addendum ☐ Waiver of Consent Documentation → Complete Appendix M3

b. Describe the consent process. Explain when and where consent will be obtained and how subjects and/or their legally authorized representatives will be provided sufficient opportunity (e.g., waiting period, if any) to consider participation.

Before the initial screening, the study will be explained individually to the participants via email. If a person expresses interest, he or she will be invited to the GCRC for initial screening. He or she will be emailed the informed consent to read before the meeting. Or, the subject can read the informed consent first thing at the initial screening. Before any measurements are taken or questionnaires...
completed, the participants will sign the informed consent. At any time the participant will be allowed to ask questions. Informed consent will thus be obtained before the sample acquisition begins (including those needed to deem eligibility). The consent form will be approved by the Institutional Review Board and will detail the purpose of the study, the procedures, the nature of the intervention, risks and benefits, confidentiality, and options for withdrawal from the study. Each subject will be informed both verbally and with the written consent document about the benefits and risks associated in participating in the study, and all questions concerning the study will be answered.

c. List the investigator(s) and/or key personnel who will obtain consent from participants or their legally authorized representatives.
Yael Vodovoz, Amber Simmons

d. Explain how the possibility of coercion or undue influence will be minimized in the consent process.

All of the participant’s questions will be answered thoroughly and truthfully. It will be explained that each subject has the choice not to participate and will be able to leave the study at any time without stating a reason for withdrawal. The amount of compensation for participation in this study was chosen on the basis of being fair amount without being so high that it might be a significant reason to participate in the study.

e. Will any other tools (e.g., quizzes, visual aids, information sheets) be used during the consent process to assist participant comprehension?

Yes \(\rightarrow\) Provide copies of these tools
No

f. Will any other consent forms be used (e.g., for clinical procedures such as MRI, surgery, etc. and/or consent forms from other institutions)?

Yes \(\rightarrow\) Provide copies of these forms
No

### 24. CAPACITY TO CONSENT

Will adult participants with limited decision-making capacity or who lack the ability to consent be recruited in this research study?

Yes
No

If Yes \(\rightarrow\) Describe the likely range of participant impairment and explain how, and by whom, the capacity to consent/assent will be determined. For adults unable to provide legally effective informed consent, indicate whether assent will be obtained; or if not, explain why not.

### 25. PRIVACY & CONFIDENTIALITY

a. Does the research require access to personally identifiable private information?

Yes
No

If Yes \(\rightarrow\) Describe the steps you will take to ensure protection of the participants’ privacy.

Patient confidentiality will be maintained. Individuals will not be identified by name or by any other personal identifying information in laboratory records, reports, or publications resulting from this study. All data will be entered into a database that can only be accessed with a password and only will be accessed by the study coordinator. All questionnaires will be kept in a locked file cabinet. Blood samples will be measured for glucose content and insulin content. These samples will be coded with participant numbers, not names, and the results will be entered into a database. The raw data will only be accessed by the study coordinator and the PIs. All electronic data will be password protected.

b. Will personal or sensitive information (e.g., relating to illegal behaviors, alcohol or drug use, sexual attitudes, mental health, etc.) be accessed or collected from participants?

Yes
No

If Yes \(\rightarrow\) Describe information.

A dietary record form could potentially solicit sensitive information if the participant is embarrassed about what they ate recently.
c. Could disclosure of information be potentially damaging to participants’ financial standing, employability or reputation, or place the participants at risk of criminal or civil liability? □ Yes ☒ No

If Yes → Explain.

d. Explain how you will protect the confidentiality of identifiable data, including where data will be stored, what security measures will be applied, and who will have access to the data.

Only the principle investigators and the research assistant will have access to the samples. The samples will be coded and not labeled with personal data. Procedures are in place to maintain confidentiality, such as password protected computer access, locked file cabinets, and rooms where records are kept.

e. Will you be obtaining a NIH Certificate of Confidentiality? □ Yes → Provide a copy before you begin the research ☒ No


f. Explain any circumstances (ethical or legal) where it would be necessary to break confidentiality. ☒ N/A

g. Indicate what will happen to the identifiable data at the end of the study. Check all that apply:

□ Identifiers separated or permanently removed from the data
☒ Identifiable/coded data is retained
□ Other, specify: __________

h. Indicate how study results might be disseminated. Check all that apply:

☒ Conference/Presentation
□ Dissertation/Thesis
□ Publication/Journal article
□ Other, specify: __________

26. HIPAA RESEARCH AUTHORIZATION

Will individually identifiable Protected Health Information (PHI) subject to the HIPAA Privacy Rule requirements be accessed, used, or disclosed in the research study? □ Yes ☒ No → Go to Question #27

If Yes → Will a written authorization be used?

☒ Yes → Provide a copy of the Authorization Form

a. Describe the PHI involved in the research (e.g., demographic information, health history, diagnosis, test results). Be as specific as possible. Provide a copy of the data collection form(s) to be used.

b. List the source(s) of the PHI (e.g., OSUMC Information Warehouse, physician’s own records, etc.), including whether any information will be obtained from sources external to OSU.

□ No → Indicate the type of waiver or alteration requested (check all that apply) and complete Appendix X.

□ Partial Waiver (recruitment purposes only)
□ Full Waiver (entire research study)
☐ Alteration (written documentation)

27. REASONABLY ANTICIPATED BENEFITS
a. List the potential benefits that participants may expect as a result of this research study. State if there are no direct benefits to individual participants. Compensation is not to be considered a benefit.

This study will most likely have minimal benefit to the individual participant. One potential benefit is glucose monitoring.

b. List the potential benefits that society and/or others may expect as a result of this research study.

Society will gain knowledge of the glycemic and insulin indexes of soy-based baked goods compared to wheat-based baked goods. This is especially important for the diabetic community and anyone that closely monitors their blood glucose.

28. RISKS, HARMS, & DISCOMFORTS
a. Indicate all reasonably expected risks/harms/discomforts that may apply to the research study:
   - ☐ Breach of confidentiality
   - ☐ Discovery of previously unknown condition
   - ☒ Economic risk
   - ☐ Invasion of privacy (participants or other individuals)
   - ☒ Physical injury or discomfort
   - ☐ Psychological stress
   - ☐ Risk to reputation
   - ☐ Social or legal risk
   - ☐ Other
   - Specify:

b. For each category of risk checked above, describe the specific risk. For physical injury or discomfort include the following:
   - Frequency/likelihood of occurrence
   - Potential severity of the harm/discomfort
   - Possible consequences (including long-term effects)

Reference the section of this application (e.g., Appendix F for drugs) if the risks are described elsewhere.

Subjects may feel an invasion of privacy when reporting their food consumption for the past 3 days. Young women, especially, may be self-conscious of their eating habits and may not want you to know what they ate. Subjects can potentially feel discomfort from the blood collection at the site of venipuncture and/or finger stick. They could also become lightheaded after the blood collection but this is unlikely due to the small amount of blood we are collecting.

There is a chance that the subjects will not tolerate the pretzel(s) or the standards and they will not be able to wash the taste out of their mouths with anything besides water.

c. Describe the specific protections that will be used to minimize the identified risks and harms.

Venipuncture and finger sticks will be performed by trained professionals. If they express a great deal of discomfort they will be reminded that they can leave at any time.

The patients will be made aware of the 3-day diet recall ahead of time.

Copious amounts of water will be provided to wash the taste out of their mouths if they do not like any of the food.

29. MONITORING

Does the research involve greater than minimal risk (i.e., are the harms or discomforts described in Question #28 beyond what is ordinarily encountered in daily life or during the performance of routine physical or psychological tests)?

☐ Yes
☐ No

If Yes → Describe the plan to oversee and monitor data collected to ensure participant safety and data integrity. Include the following:
   - The information that will be evaluated (e.g., incidence and severity of actual harm compared to that expected);
     - Who will perform the monitoring (e.g., investigator, sponsor, or independent monitoring committee);
   - Timing of monitoring (e.g., at specific points in time, after a specific number of participants have been enrolled); and
• Decisions to be made as a result of the monitoring process (e.g., provisions to stop the study early for unanticipated problems).

30. ASSESSMENT OF RISKS & BENEFITS
Discuss how risks to participants are reasonable when compared to the anticipated benefits to participants (if any) and the importance of the knowledge that may reasonably be expected to result.

Risk for the participants will be minimal, and the participants will be compensated $50 per visit. Most likely, participants will not have tried a soy-based baked good before and will be granted this special opportunity. We will closely monitor every participant and will immediately discontinue the study if we see any signs of toxicity or allergic reactions. (This is particularly important for those who have never had soy and/or have other allergies.) Knowledge gained from this study will supplement the currently limited knowledge pool on soy products. It could potentially change how blood glucose monitors view soy products and could potentially influence the direction of food companies that heed glycemic and/or insulin indexes.

31. ALTERNATIVES TO STUDY PARTICIPATION
Other than choosing not to participate, list any specific alternatives, including available procedures or treatments that may be advantageous to the subject.
None.

32. PARTICIPANT COSTS/REIMBURSEMENTS
a. List any potential costs subjects (or their insurers) will incur as a result of study participation (e.g., parking, study drugs, diagnostic tests, etc.).

We will provide the pretzels and glucose standards and we will also provide parking passes for any study related visit.

b. List any costs to participants that will be covered by the research study.
The cost to produce and distribute the pretzels, the time and effort of the study coordinators, all blood analyses, and all future data analyses will be covered by the research study and the GRC.

33. APPLICATION CONTENTS
Indicate what documents are being submitted for this research project. Check all appropriate boxes and provide the version number and date, if available.

- Initial Review of Human Subjects Research Application
  Version: 2.0 Date: 10/06/08
- Appendix A1: OSU Co-Investigators & Key Personnel (questions 4 & 5) Version: 2.0 Date: 10/06/08
- Appendix A2: External (non-OSU) Co-Investigators & Key Personnel (question 6)
- Appendix B: Expedited Review – Initial Review (question 13)
- Appendix C: Data Repositories (question 17b)
- Appendix D: Deception (question 17b)
- Appendix E: Devices (question 17b)
- Appendix F: Drugs or Biologies (question 17b)
- Appendix G: Genetic Testing (question 17b)
- Appendix H: Storage of Biological Materials (question 17b)
- Appendix I: Children (question 20b)
- Appendix J: Non-English Speaking Participants (questions 20b and 23a)
- Appendix K: Pregnant Women/Fetuses/Neonates (question 20b)
34. ASSURANCE

PRINCIPAL INVESTIGATOR (or Advisor)

I agree to follow all applicable policies and procedures of The Ohio State University and federal, state, and local laws and guidance regarding the protection of human subjects in research, as well as with professional practice standards and generally accepted good research practice guidelines for investigators, including, but not limited to, the following:

- The research will be performed as approved by the IRB under the direction of the Principal Investigator (or Advisor) by appropriately trained and qualified personnel with adequate resources;
- The research will not be initiated until written notification of IRB approval has been received;
- Informed consent and HIPAA research authorization from human subjects (or their legally authorized representatives) will be obtained and documented (unless waived) prior to their involvement in the research using the currently IRB-approved consent form(s) and process;
- Promptly report to the IRB events that may represent unanticipated problems involving risks to subjects or others;
- Significant new findings that develop during the course of the study that may affect the risks or benefits of participation will be reported;
- The IRB will be informed of any proposed changes in the research or informed consent process before changes are implemented, and no changes will be made until approved by the OSU IRB (except where necessary to eliminate apparent immediate hazards to participants);
- A Continuing Review of Human Subjects Research application will be completed and submitted before the deadline for review at intervals determined by the IRB to be appropriate to the degree of risk (but not less than once per year) to avoid expiration of IRB approval and cessation of all research activities;
- Research-related records (and source documents) will be maintained in a manner that documents the validity of the research and integrity of the data collected, while protecting the confidentiality of the data and privacy of participants;
- Research-related records will be retained and available for audit for a period of at least three years after the research has ended (or
longer, according to sponsor or publication requirements) even if I leave the University;

- The Office of Responsible Research Practices will be contacted for assistance in amending (to request a change in Principal Investigator) or terminating the research if I leave the University or am unavailable to conduct or supervise the research personally (e.g., sabbatical or extended leave);
- A Final Study Report will be provided to the IRB when all research activities have ended (including data analysis with individually identifiable or coded private information); and
- All Co-Investigators, research staff, employees, and students assisting in the conduct of the research will be informed of their obligations in meeting the above commitments.

I verify that the information provided in this Initial Review of Human Subjects Research application is accurate and complete.

Signature of Principal Investigator (or Advisor)  Date

Printed name of Principal Investigator (or Advisor)

DEPARTMENT CHAIR (or Signatory Official)

As Department Chair (or Signatory Official) for the Principal Investigator, I acknowledge that this research is in keeping with the standards set by our unit and that it has met all Departmental/College requirements for review.

If the PI or any Co-Investigator is also the Department Chair, the signature of the Dean or other appropriate Signatory Official, such as the Associate Dean for Research, must be obtained.

Signature of Department Chair  Date

Printed name of Department Chair
# APPENDIX A1

## OSU Co-Investigators & Key Personnel

Complete this form to list OSU Co-Investigators and key personnel on the research study. Signatures are required of all OSU Co-Investigators. Use Appendix A2 to list external (non-OSU) Co-Investigators and key personnel.

Key personnel are defined as individuals who participate in the design, conduct, or reporting of human subjects research. At a minimum, include individuals who recruit or consent participants or who collect study data.

All OSU individuals listed on this protocol will have access to information about IRB actions and the completion status of each individual’s administrative and training requirements (CTTI, COI disclosure). Note: Personal financial information provided in COI disclosures is not included.

**PI Name:** Yael Vodovotz

## OSU CO-INVESTIGATORS

As Co-Investigator, I agree to comply with all policies and procedures of The Ohio State University and federal, state, and local laws and guidance regarding the protection of human subjects in research, as well as with professional practice standards and generally accepted good research practice guidelines for investigators.

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<tr>
<th>Name (Last, First, MI):</th>
<th>Miller, Carla K</th>
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<td>Associate Professor</td>
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<td>Human Nutrition</td>
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<td>Phone:</td>
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<tr>
<td>E-mail:</td>
<td><a href="mailto:cmiller@ehe.osu.edu">cmiller@ehe.osu.edu</a></td>
</tr>
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**Signature of Co-Investigator**

**Date**

**Printed name of Co-Investigator**

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**Name (Last, First, MI):**

**University Academic Title:**

**Department Name (TU):**

**Department #: (TU):**

**OSU ID Number:**

**Degree(s):**

**College (TU):**

**Phone:**

**E-mail:**

**Signature of Co-Investigator**

**Date**

**Printed name of Co-Investigator**

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**Name (Last, First, MI):**

**University Academic Title:**

**Department Name (TU):**

**Department #: (TU):**

**OSU ID Number:**

**Degree(s):**

**College (TU):**

**Phone:**

**E-mail:**

**Signature of Co-Investigator**

**Date**

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Page 1 of 3

Form date: 10/06/08

Version 2.0
## OSU CO-INVESTIGATORS

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<th>Simmons, Amber L</th>
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<th>Human Nutrition</th>
<th>University Title:</th>
<th>Graduate Student</th>
<th>OSU ID Number:</th>
<th>05158616</th>
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APPENDIX B

Expeditied Review – Initial Review

Complete this form to request expedited review of the proposed research. If the research meets the conditions for expedited review, the review of the protocol will be carried out by the IRB chairperson or by one or more experienced reviewers designated by the chairperson from among members of the IRB.

See 45 CFR 46 and 21 CFR 56 for more information.

PI Name: Yael Vodovotz

Conditions required for expedited IRB review:

1) The Federal Regulations establish two main criteria for an expedited review:
   a) The research may not involve more than "minimal risk." "Minimal risk" means that "the probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." (45 CFR 46.102(i) and 21 CFR 56.102(i)).
   b) The entire research project must be consistent with one or more of the federally defined categories.

2) The categories in this list apply regardless of the age of the participants, except as noted. The expedited review procedure may not be used where identification of the participants and/or their responses would reasonably place them at risk of criminal or civil liability or be damaging to the participant's financial standing, employability, insurability, reputation, or be stigmatizing, unless reasonable and appropriate protections will be implemented so that risks related to invasion of privacy and breach of confidentiality are no greater than minimal.

3) The expedited review procedure may not be used for classified research involving human subjects or research involving prisoners as participants.

4) Investigators and IRBs are reminded that the standard requirements for informed consent (or its waiver, alteration, or exception) apply regardless of the type of review (i.e., expedited or convened) utilized by the IRB.

Select the category that best describes the research project.

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<tr>
<td>(1)</td>
<td>Clinical studies of drugs and medical devices only when condition (a) or (b) is met.</td>
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<tr>
<td></td>
<td>a) Research on drugs for which an investigational new drug application (21 CFR 312) is not required. (Note: Research on marketed drugs that significantly increases the risks or decreases the acceptability of the risks associated with the use of the product is not eligible for expedited review.)</td>
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<td></td>
<td>b) Research on medical devices for which (i) an investigational device exemption application (21 CFR 812) is not required; or (ii) the medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.</td>
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<p>| (2) | Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows: |
|     | a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these participants, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week. |
|     | b) from other adults and children (defined as &quot;persons who have not attained the legal age for consent to treatments or procedures involved in the research, under the applicable law of the jurisdiction in which the research will be conducted.&quot; 45 CFR 46.402(a)), considering the age, weight, and health of the participants, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these participants, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week. |</p>
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| (3) | Prospective collection of biological specimens for research purposes by non-invasive means.  
**Examples:** (a) hair and nail clippings in a nondisfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) unaccommodated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax or by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane prior to or during labor; (h) supra- and subgingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization. |
| (4) | Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)  
**Examples:** (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the participant or an invasion of the participant's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual. |
| (5) | Research involving materials (data, documents, records, or specimens) that have been collected or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.) |
| (6) | Collection of data from voice, video, digital or image recordings made for research purposes. |
| (7) | Research made on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.) |
APPENDIX T
All Other Research Changes

Complete this form to request changes to IRB approved research, except changes in study personnel or participant numbers. Use Appendix D to request changes in study personnel and Appendix O to request changes in participant numbers.

As applicable, provide the currently approved materials (marked as “current”), and the revised materials, one copy with change(s) underlined (or “tracked”) and one copy with change(s) incorporated (clean). All materials should be submitted single sided.

To request changes before the next continuing review, include the "Amendment/Changes to Research" form, found at http://orpp.osu.edu/irb-umend/index.cfm.

1. Describe the change(s) to the research and provide a rationale for each change.
Based on the established ideal protocol for glycemic index testing, we planned to collect blood via finger-sticks, 7/day, for glucose concentration determination. This has been shown to provide the smallest standard deviations in glycemic index determination. However, we did not realize that this would require one stick per finger and may inhibit patients’ abilities to perform routine activities later in the day such as typing or cross-stitching. We are already collected blood via venous catheter for insulin concentration measurements, so we decided that it would be gentler on the patients to collect the blood for the glucose assay through the catheter also. We are compromising the accuracy of the glucose concentration because capillary blood is more accurate than venous blood, however, we may improve patient compliance.

2. Will there be any change in the risk(s) to participants?

☐ Yes
☐ No

If Yes ➔ Explain: There is now slightly less risk than before because we are not doing finger-stick blood collection.

3. Will there be any change in the benefit(s) to participants? Compensation is not to be considered a benefit.

☐ Yes
☐ No

If Yes ➔ Explain:

4. Will the proposed change(s) affect participants’ willingness to take part in the research?

☐ Yes
☐ No

If Yes ➔ How will information be communicated to currently enrolled subjects (e.g., revised consent form, letter to participants, etc.)? No one is enrolled yet. This may lead participants to be slightly less hesitant to participate.
Appendix L: Recruitment Flyer for Glycemic Index and Insulinemic Index Study
Soy Pretzel Study

**WHO:**
Healthy nonsmokers without history of diabetes, glucose intolerance, or gastrointestinal disorders.

**WHAT:**
We are determining the glycemic index of a soy-based soft pretzel compared to a wheat pretzel. Subjects will consume a wheat pretzel, a soy pretzel, white bread, and a glucose standard over the course of 6 visits. Blood will be drawn before you consume the snack and over two hours following your first bite.

**WHY:**
Foods that have a high glycemic index cause rapid fluctuations in blood sugar and insulin that can contribute to long term health risks such as diabetes and heart disease. There is a drive to develop more healthful versions of the foods that we love in order to make it easier for Americans to have control over this blood sugar.

**RISKS AND BENEFITS:**
You will be one of the first people in America to try the new soft pretzel! Participants will be paid $50 per visit for a maximum of $300 in compensation of their time. It is unlikely that you will receive any significant risks from participating in this study.

**HOW:**
If you or any one you know might be interested, please contact Amber Simmons at simmons.225@osu.edu for more information.
Appendix M: Consent Form for Glycemic Index and Insulinemic Index
The Ohio State University Consent to Participate in Research

Study Title: Determination of the glycemic and insulin indexes of a soy pretzel in healthy individuals

Principal Investigator: Yael Vodovotz, Ph.D.

Sponsor: Ohio Agricultural Research and Development Center

• This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.

• Your participation is voluntary. You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

• You may or may not benefit as a result of participating in this study. Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor.

• You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

1. Why is this study being done?

This study is being performed to determine the blood glucose (blood sugar) and blood insulin response of a soy soft pretzel compared to a wheat soft pretzel. A lower blood glucose and insulin response after eating are associated with better health outcomes.

2. How many people will take part in this study?

You will be one of 10-12 participants. Participants will be male or female healthy adults age 18-45.

Female participants cannot be pregnant or lactating and must not plan on getting pregnant within the duration of the study. These exclusion criteria exist due to potential effects on
glucose metabolism and requirements for fasting. A simple yes or no question will
determine whether a woman is pregnant or not.

3. What will happen if I take part in this study?

You will be screened initially for fasting glucose level. You will fill out a health
questionnaire for information on medical conditions and use of medications or dietary
supplements that could affect your glucose tolerance or food intake. We will also measure
your height and weight.

The glycemic index (GI) will be determined on a soy soft pretzel and wheat soft
pretzel as well as glucose and white bread. You will test each of the pretzels and white
bread in random order. In addition, you will be required to complete a total of 3
replications with the glucose standard as is recommended for GI standardization. Thus,
you will complete a total of 6 treatment visits (2 trials for the pretzels, 1 trial for the bread,
and 3 trials with the glucose standard).

For 3 days prior to each treatment visit, you will be asked to complete dietary record
forms to insure that a carbohydrate intake of at least 150 g/d was eaten. (This is a normal
amount of carbohydrates. You do not need to closely monitor your carbohydrate intake
yourself unless you think you do not consume a normal carbohydrate intake on a given
day.) On the night before the treatment visit, you will be asked to begin fasting after the
evening meal (no food or beverages except water for at least 10 hours). Upon arrival to the
laboratory the next morning, we will record your temperature and blood pressure and you
will rest quietly for 30 min- you may want to bring a book or magazine. If dietary records
indicate low carbohydrate intake or if temperature or blood pressure are not within normal
range, the treatment visit will be rescheduled. During the 30 min rest period, intravenous
soft tube (catheter) will be placed in the upper arm in a vein to obtain blood samples.
After the completion of the 30 min rest period, baseline blood glucose and insulin
concentrations will be measured.

Immediately thereafter, you will be fed a meal consisting of a portion size of the test
food that is calculated from the food label to provide 50 g available carbohydrate (total
carbohydrate minus dietary fiber) and 8 ounces of water. This is about 2 small soft
pretzels or about 2 slices of bread. The glucose standard will consist of an orange-
flavored test beverage that contains 50 g glucose in 30 mL of water. Timing of subsequent
blood sampling will start with the first bite of the test meal. You will resume fasting (sips
of water only) after the test meal. Additional blood samples for glucose and insulin
responses will be obtained at 15, 30, 45, 60, 90, and 120 min after the start of the test meal
through the catheter. Only minimal physical activity will be allowed during the blood
testing. A minimum of 96 hours will separate each treatment visit.

4. How long will I be in the study?

You will need to come in for 6 visits besides the initial screening. These visits need to be
at least 96 hours (4 days) apart. It is easiest if we schedule one visit per week. The
average participant will be in the study for 6 weeks.
5. Can I stop being in the study?

You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your future relationship with The Ohio State University.

6. What risks, side effects or discomforts can I expect from being in the study?

You will be having blood drawn through both a soft tube in your arm.

7. What benefits can I expect from being in the study?

There are no known benefits. The findings from this study will help us to better evaluate the potential health benefits of the soy pretzel.

8. What other choices do I have if I do not take part in the study?

You may choose not to participate without penalty or loss of benefits to which you are otherwise entitled. There are no alternatives to participate in the study.

9. Will my study-related information be kept confidential?

Efforts will be made to keep your study-related information confidential. However, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law. Also, your records may be reviewed by the following groups (as applicable to the research):

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- U.S. Food and Drug Administration;
- The Ohio State University Institutional Review Board or Office of Responsible Research Practices;
- The sponsor supporting the study, their agents or study monitors; and
- Your insurance company (if charges are billed to insurance).

If the study involves the use of your protected health information, you may also be asked to sign a separate Health Insurance Portability and Accountability Act (HIPAA) research authorization form.
10. What are the costs of taking part in this study?

The blood draw may hurt. There may be slight bruising around the blood site. However, trained personnel will take the blood draw. Personnel at the General Clinical Research Center are trained in taking blood samples to minimize pain and bruising.

11. Will I be paid for taking part in this study?

You will receive $50 cash at each visit for a total of $300. By law, payments to subjects are considered taxable income. The payments will be pro-rated in case you choose to withdraw from the study early.

12. What happens if I am injured because I took part in this study?

If you suffer an injury from participating in this study, you should notify the researcher or study doctor immediately, who will determine if you should obtain medical treatment at The Ohio State University Medical Center.

The cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

13. What are my rights if I take part in this study?

If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study.

You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study.

You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.

An Institutional Review Board responsible for human subjects research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

14. Who can answer my questions about the study?

For questions, concerns, or complaints about the study you may contact Amber Simmons at (614) 247-7686 or Yael Vodovotz at (614) 247-7696
For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251.

If you are injured as a result of participating in this study or for questions about a study-related injury, you may contact Yael Vodovotz at (614)247-7696.
**Consign the consent form**

181 I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.

187 I am not giving up any legal rights by signing this form. I will be given a copy of this form.

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192 Investigator/Research Staff

193 I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

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<th>Printed name of person obtaining consent</th>
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Appendix N: HIPAA Form for Glycemic Index and Insulinemic Index Study

THE OHIO STATE UNIVERSITY
AUTHORIZATION TO USE
PERSONAL HEALTH INFORMATION IN RESEARCH

Title of the Study: Determination of the glycemic and insulin indexes of a soy pretzel in healthy individuals
OSU Protocol Number: 2009H00075
Principal Investigator: Yael Vodovotz

Subject Name ____________________________

Before researchers use or share any health information about you as part of this study, The Ohio State University is required to obtain your authorization. This helps explain to you how this information will be used or shared with others involved in the study.

• The Ohio State University and its hospitals, clinics, health-care providers and researchers are required to protect the privacy of your health information.

• You should have received a Notice of Privacy Practices when you received health care services here. If not, let us know and a copy will be given to you. Please carefully review this information. Ask if you have any questions or do not understand any parts of this notice.

• If you agree to take part in this study your health information will be used and shared with others involved in this study. Also, any new health information about you that comes from tests or other parts of this study will be shared with those involved in this study.

• Health information about you that will be used or shared with others involved in this study may include your research record and any health care records at the Ohio State University. For example, this may include your medical records, x-ray or laboratory results. Psychotherapy notes in your health records (if any) will not, however, be shared or used. Use of these notes requires a separate, signed authorization.

Please read the information carefully before signing this form. Please ask if you have any questions about this authorization, the University’s Notice of Privacy Practices or the study before signing this form.

Initials/Date: _________________________
Those Who May Use, Share And Receive Your Information As Part Of This Study

- Researchers and staff at The Ohio State University will use, share and receive your personal health information for this research study. Other Ohio State University staff not involved in the study but who may become involved in your care for study-related treatment will have access to your information.

- Those who oversee the study will have access to your information, including:
  - Members and staff of the Ohio State University’s Institutional Review Boards, including the Western Institutional Review Board
  - The Office for Responsible Research Practices
  - University data safety monitoring committees
  - The Ohio State University Research Foundation

- Your health information may also be shared with federal and state agencies that have oversight of the study or to whom access is required under the law. These may include:
  - The Food and Drug Administration
  - The Office for Human Research Protections
  - The National Institutes of Health
  - The Ohio Department of Human Services

These researchers, companies and/or organization(s) outside of The Ohio State University may also use, share and receive your health information in connection with this study:

None.

The information that is shared with those listed above may no longer be protected by federal privacy rules.

Initials/Date ______________

Page 2 of 3
Authorization Period

This authorization will not expire unless you change your mind and revoke it in writing. There is no set date at which your information will be destroyed or no longer used. This is because the information used and created during the study may be analyzed for many years, and it is not possible to know when this will be complete.

Signing the Authorization

- You have the right to refuse to sign this authorization. Your health care outside of the study, payment for your health care, and your health care benefits will not be affected if you choose not to sign this form.
- You will not be able to take part in this study and will not receive any study treatments if you do not sign this form.
- If you sign this authorization, you may change your mind at any time. Researchers may continue to use information collected up until the time that you formally changed your mind. If you change your mind, your authorization must be revoked in writing. To revoke your authorization, please write to:
  [insert name and contact information for the Principal Investigator]
  or [insert contact information for the appropriate HIPAA Privacy Contact].
- Signing this authorization also means that you will not be able to see or copy your study-related information until the study is completed. This includes any portion of your medical records that describes study treatment.

Contacts for Questions

- If you have any questions relating to your privacy rights, please contact [insert contact information for the appropriate HIPAA Privacy Contact].
- If you have any questions relating to the research, please contact [insert name and contact information for the Principal Investigator].

Signature

I have read (or someone has read to me) this form and have been able to ask questions. All of my questions about this form have been answered to my satisfaction. By signing below, I permit [insert name of Principal Investigator] and the others listed on this form to use and share my personal health information for this study. I will be given a copy of this signed form.

Signature
(Subject or Legally Authorized Representative)

Name
(Print name above)
(If legal representative, also print relationship to subject.)

Date__________ Time _________ AM / PM
Appendix O: Ohio State University Institutional Review Board Approval
September 17, 2009

Protocol Number:  2009H0075
Protocol Title:   DETERMINATION OF THE GLYCEMIC AND INSULIN INDEXES OF A SOY PRETZEL IN HEALTHY INDIVIDUALS, Yuel Vodovotz, Carla K. Miller, Food Science & Technology

Type of Review:  Initial Review – expedited
IRB Staff Contact:  Kim Kovarik
614-262-9804
Kovarik.9@osu.edu

Dear Dr. Vodovotz,

The Biomedical IRB APPROVED BY EXPEDITED REVIEW the above referenced research. The Board was able to provide expedited approval under 45 CFR 46.110(b)(1) because the research meets the applicability criteria and one or more categories of research eligible for expedited review, as indicated below.

Date of IRB Approval:  September 17, 2009
Date of IRB Approval Expiration:  April 7, 2010
Expedited Review Category:  2

If applicable, informed consent (and HIPAA research authorization) must be obtained from subjects or their legally authorized representatives and documented prior to research involvement. The IRB-approved consent form and process must be used. Changes in the research (e.g., recruitment procedures, advertisements, enrollment numbers, etc.) or informed consent process must be approved by the IRB before they are implemented (except where necessary to eliminate apparent immediate hazards to subjects).

This approval is valid for one year from the date of IRB review when approval is granted or modifications are required. The approval will no longer be in effect on the date listed above as the IRB expiration date. A Continuing Review application must be approved within this interval to avoid expiration of IRB approval and cessation of all research activities. A final report must be provided to the IRB and all records relating to the research (including signed consent forms) must be retained and available for audit for at least 3 years after the research has ended.

It is the responsibility of all investigators and research staff to promptly report to the IRB any serious, unexpected and related adverse events and potential unanticipated problems involving risks to subjects or others.

This approval is issued under The Ohio State University’s OHRP Federalwide Assurance #00006378.

All forms and procedures can be found on the ORRP website – www.orrp.osu.edu. Please feel free to contact the IRB staff contact listed above with any questions or concerns.

Karla Zadnik, OD, PhD, Chair
Biomedical Institutional Review Board

In-017-06 Exp Approval New CR
Version 08/11/09
References


A. Marangoni, (2011) “Everything you wanted to know about lipid polymorphism, but were afraid to ask,” in 102nd AOCS Annual Meeting & Expo, Cincinnati, OH.


