

# Development and Characterization of a Soy-Based Soft Pretzel Designed for Exercise Recovery

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## Introduction

High intensity exercise can lead to exercise-induced muscle damage (EIMD),<sup>1</sup> a term used to describe the loss of strength, soreness, swelling, reduced range of motion, and release of muscular enzymes and proteins that follow exercise<sup>2</sup>. Muscle damage triggers an immune response that leads to production of reactive oxygen and nitrogen species (RONS)<sup>2</sup>. RONS are important for the breaking down and removal of cell debris, but their accumulation in muscle following strenuous exercise contributes to inhibition of muscle strength and soreness<sup>3-5</sup>. Reducing or blocking inflammation can interfere with normal muscle adaptation, discouraging the use of long-term supplementation with anti-inflammatory or antioxidant compounds<sup>2</sup>. However, in the case where athletes or professionals (i.e. firefighters, military) are performing multiple bouts of intense exercise with little recovery time in between, an acute or periodized approach to antioxidant or anti-inflammatory compound supplementation may be beneficial in accelerating recovery and reducing symptoms<sup>1</sup>. Protein and carbohydrates have also been recommended for muscle recovery due to their influence on glycogen replenishment and the rate of muscle protein synthesis<sup>6,7</sup>. Antioxidant compounds<sup>8</sup>, phytochemicals<sup>9,10</sup>, protein<sup>11-15</sup>, carbohydrates<sup>8</sup> and combinations thereof<sup>16</sup> have been utilized to enhance muscle recovery.

Soybeans contain isoflavones and other phytochemicals, which have been shown to reduce inflammation in both exercise and disease states<sup>9,10,17</sup>. Soy flour contains around 50% protein by mass and high levels of isoflavones<sup>18</sup>. The inclusion of soy flour in a food product

offers a combined strategy for exercise recovery, including anti-inflammatory isoflavones, protein, and carbohydrates.

Bread has been used as a vehicle for soy ingredients in past studies with varying degrees of success.<sup>19-22</sup> Up to 50% replacement of wheat flour with soy protein ingredients and vital wheat gluten have been attempted, resulting in bread products with lower loaf volume and a more dense texture than conventional wheat bread<sup>22,23</sup>. While these texture attributes may not be desirable in bread, a chewier and denser crumb is a common feature of soft pretzels. Previous studies performed by this lab group have successfully added soy ingredients to soft pretzels for applications with satiety and obesity<sup>19,24</sup>. While these products were developed for different applications and were lower in protein than is recommended for exercise recovery, the studies show the potential for soy addition to soft pretzels. This and the pretzel's popularity as a snack food, led to its selection as a vehicle for soy ingredients, targeted for exercise recovery.

There are challenges to formulating with soy ingredients due to the possibility of "beany" or bitter flavors. "Beany" flavor compounds are produced by the oxidation of oils by endogenous lipoxygenases and are thought to be undesirable<sup>25</sup>. Bitterness of soy ingredients is derived through at least two mechanisms. Soy proteins are sometimes hydrolyzed to improve functionality in foods, which can lead to the formation of bitter peptides<sup>26</sup>. Additionally, isoflavones and other polyphenols in soybeans such as soyasaponins, also have a bitter flavor<sup>27</sup>. Soft pretzels have a characteristic flavor from the lye or other alkaline solution used in their production<sup>28</sup>. The relatively strong and, to some extent, bitter flavor of pretzels may mask the off flavors of soybeans.

Clinical trials utilizing functional foods have distinct challenges. In food selection, taste, followed by affordability and nutritional value, was found to be the most important attribute in a Mintel survey of 2000 adults<sup>29</sup>. Additionally studies have found that consumers are not willing to sacrifice taste for health benefits in functional foods<sup>30</sup>. So, while nutritional value is important in food selection, it is not the only factor to consider. Functional food acceptability has also been shown to influence compliance in clinical studies<sup>31</sup>. The goal of this study was to develop and characterize a high protein soy soft pretzel that was similar in structure to a traditional pretzel, acceptable in the target population, and appropriate for use in future clinical trials focused on exercise recovery.

## Materials and Methods

### *Pretzel Ingredients and Preparation*

Soy pretzels were prepared using high gluten wheat flour (Bay State Milling; Quincy, MA), defatted soy flour (Archer Daniels Midland; Chicago, IL), soymilk powder (Devansoy; Rock City, IL), vital wheat gluten (Hodgson Mill; Effingham, IL), Domino granulated sugar (American Sugar Refining; West Palm Beach, FL), Crisco vegetable oil (The J.M. Smucker Company, Orrville, OH) Diamond Crystal Kosher salt (Cargill; Wayzata, MN), SAF instant yeast (Lesaffre; Marcq-en-Barœul, France), and Gem 100 dough conditioner (flour, enzymes, ascorbic acid) (Caravan; Lenexa, KS). Pretzel salt (Cargill; Wayzata, MN) was used to top the pretzels for sensory evaluation only. Conventional wheat pretzels were prepared using the above ingredients with the exception of soy flour, soymilk powder, and gluten. The approximate formulations as a baker's percentage (ingredients expressed as a percentage of flour in wheat pretzel) are listed in Table 1. The soy pretzels required greater hydration than wheat pretzels due to the greater water holding capacity of soy flour<sup>32</sup>. Therefore, the soy pretzels used 81%

hydration while the wheat pretzels use 60% hydration. Additional vegetable oil was also added to the wheat pretzel formula to account for the larger lipid concentration in the soymilk powder. The two formulas were chosen to be isocaloric and contain the same concentration of lipids to meet the requirements of future clinical studies.

*Table 1: Pretzel Formulations*

Ingredient	Composition (Baker's Percent)	
	Wheat Pretzel	Soy Pretzel
Wheat Flour	100	42.33
Soy Flour	0	32.76
Soymilk Powder	0	10.92
Vital Wheat Gluten	0	13.40
Granulated Sugar	3.88	3.88
Vegetable Oil	6.60	3.88
Salt	1.36	1.36
Instant Yeast	1.94	1.94
Dough Conditioner	1.94	1.94
Water	60.00	81.17

Pretzels were prepared in 1 kg batches as follows. All ingredients were added to a Hobart mixer (Illinois Tool Works; Glenview, IL). Using the dough hook attachment, the ingredients were mixed on low for 7 minutes. The dough was bulk proofed for 1 hour at room temperature. Dough was then shaped into 17-gram balls for wheat and 20-gram balls for soy pretzels to create pretzel balls of similar caloric content. Wheat pretzels were smaller to account for the reduced water in the formula. The shaped pretzel balls were proofed for an additional 1 hour at room temperature before being dipped in a 5% lye solution for wheat pretzels and a 3% lye solution for soy pretzels. The solution was made from food grade sodium hydroxide (Bulk Apothecary; Aurora, OH) and distilled water. Lye concentration differed to produce pretzels that were more similar in color. The pretzel balls were baked for 11 minutes at 165°C (300°F). For sensory

testing only, cooled pretzels were coated with a thin layer of oil and topped with a small amount of pretzel salt.

#### *Loaf Volume and Specific Volume*

Loaf volume was measured using rapeseed displacement according to AACC method nr 10-05 (AACC 2000). Specific volume was calculated as the loaf volume divided by the pretzel mass.

#### *Moisture loss on baking*

A sample of pretzels (5 replicates per sample type) were weighed immediately before dipping in the lye solution and immediately after baking to determine the percentage of moisture loss from the pretzel after baking.

#### *Thermogravimetric analysis (TGA)*

The moisture contents and desorption patterns were measured using a Thermogravimetric Analyzer Model Q5000 (TA Instruments, New Castle, DE). Samples of 10-15 mg of pretzel crumb were placed in aluminum pans (TA Instruments) and heated from room temperature to 200°C at a linear rate of 10°C/min. The percent weight loss and derivative weight loss were analyzed in each sample using TRIOS software (version 4.5.0). The percent sample weight at 180°C was subtracted from 100% to yield the moisture content of the sample, under the assumption that all weight lost was from moisture. The derivative weight loss was the rate of weight loss as a function of temperature. The temperature at the peak of the derivative weight loss curve indicated when the sample experienced the greatest moisture loss. Analysis was performed in triplicate.

### *Differential scanning calorimetry (DSC)*

Calorimetry was performed using a Q100 Differential Scanning Calorimeter with a refrigerated cooling system, calibrated with indium (TA Instruments, New Castle, DE). DSC was used to calculate the amount of “freezable” and “un-freezable” water in the samples. Samples of 10-15 mg of pretzel crumb were placed in hermetically sealed aluminum pans, with an empty sealed pan used as reference. Each sample was cooled to -50°C and held isothermally for 2 minutes. The temperature was increased linearly to 150°C at a rate of 5°C/min. The peak that occurred near 0°C was associated with the phase transition from ice to water and was integrated to yield the change in enthalpy. The amount of freezable water was calculated using the latent heat of fusion of water (333 J/g)<sup>19</sup>. The amount of freezable water was subtracted from the moisture content determined by TGA to yield the amount of un-freezable water.

### *Texture Profile Analysis*

Texture analysis was performed using an Instron Universal Texture Analyzer 5542 (Instron, Norwood, MA). Pretzel pieces were subjected to a 40% double compression test using a crosshead speed of 100 mm/min<sup>19</sup>. Springiness, and chewiness were calculated using Bluehill 2 software, version 2.17 (Instron, Norwood, MA). Hardness was the maximum force on the compression head during the first compression.

### *Scanning Electron Microscopy (SEM)*

The pretzel crumb structure was observed using SEM with an FEI Apreo LoVac Analytical SEM with a low vacuum detector (Thermo Fisher Scientific, Waltham, MA). Pretzel samples were stored in an airtight container for two days at room temperature prior to analysis. A thin slice (approximately 5 mm) was cut from the center of each pretzel piece using a metal scalpel. The slice was cut into a square (approximately 1 cm<sup>2</sup>). Each pretzel sample was mounted

onto a stub using double sided carbon or copper tape. The chamber was pumped to 50 Pa. The magnifications used were 100x and 1600x.

### *Isoflavone Quantification*

Isoflavone extraction and quantification were performed using methods adapted from Morris, 2018<sup>33</sup>. Each pretzel piece was ground using a Magic Bullet Blender model MB1001 (Capital Brands, Los Angeles, CA). Approximately 0.5 g of the pretzel was placed in a 15 mL conical tube, along with 2.5 mL of 66% aqueous acetonitrile. The mixture was vortexed for 30 seconds and then sonicated for 30 minutes using a sonicator (FS30H, Fisher Scientific, Waltham, MA). The tubes were then centrifuged for 30 minutes at 3000 rpm at 15°C. The supernatant was collected in a 22 mL glass vial. This process of adding 66% acetonitrile through supernatant collection was repeated two more times. The combined supernatant from each round was vortexed briefly and a 2 mL aliquot was transferred to a clean 22 mL glass vial. The aliquot was dried under nitrogen until a thin film remained. The dried extract was stored in a -80°C freezer prior to analysis.

Previously dried samples were allowed to reach room temperature and were then resuspended in 1 mL of 80% aqueous methanol. The samples were briefly vortexed then sonicated for 15 minutes. The extract was filtered using a nylon 0.22 µm filter (Grace Discovery Sciences, Columbia, MD) into an HPLC vial.

An Agilent 110 Series HPLC unit with an autosampler and photodiode array detector (PDA) was used for separation and analysis. Reverse phase HPLC was performed using a C18 column (Symmetry, 3.5µm, 4.6 mmx 75 mm, Waters, Milford, MA). The injection volume was 10 µL. Solvent A was 0.1% formic acid in water and Solvent B was 0.1% formic acid in

acetonitrile. The solvent percentages were changed linearly using the conditions previously optimized and shown in Table 2 below.

*Table 2: HPLC Conditions for quantification of soy isoflavones*

Time (min)	Solvent A (%)	Solvent B (%)	Flow Rate (mL/min)
0	90	10	1.3
1	90	10	1.0
20	65	35	1.0
22	0	100	1.0
25	90	10	1.0
30	90	10	1.0

The standard curve was obtained using procedures previously described by Morris, 2018<sup>33</sup>.

### *Sensory Evaluation*

Overall and attribute acceptability was assessed using sensory evaluation of the soy pretzels using a protocol classified as exempt by the Ohio State University human subjects Institutional Review Board (2019E0737). The population consisted of 100 panelists (40 male and 60 female) that were students or employees of Ohio State. Fourteen of the panelists were considered athletes who were part of university or club sports teams. Sensory testing occurred in individual sensory booths. Panelists were given 2 soy pretzel pieces and then completed a survey with questions related to acceptability, appropriateness, and their diet and exercise habits. The full survey may be found in Appendix A. A 9-point hedonic scale (1=dislike extremely, 9=like extremely) was used to assess the overall, flavor, texture, and appearance acceptability. A 5-Point Just About Right (JAR) scale was used to assess specific attributes including crust color, crumb color, piece size, serving size, yeast aroma, sweetness, saltiness, bitterness, hardness, and dryness (1=much too little of the attribute, 5=much too much of the attribute). A 10-point scale was also used to determine the level of “beany” aroma and flavor in the sample. Beany aroma



and flavor was defined as the smell or taste of raw or cooked beans such as soybeans or green beans. Appropriateness was assessed using a check all that apply question concerning when the panelist would consume the product. The frequencies of exercise and high protein product consumption were also assessed.

Due to the limited participation by athletes in the lab setting, sensory evaluation was also performed with a group of collegiate rugby players in an on field setting. Evaluation occurred before practice on the rugby field to better mimic the conditions in which the product would be consumed. By conducting the study before practice instead of after, the impact of previous physical activity was minimized. The same survey used in the previous sensory study was used in this group. Sensory evaluation on the practice field is further referred to as “field”. The protocol was approved as exempt by the Ohio State Institutional Review Board (Protocol 2019E0893).

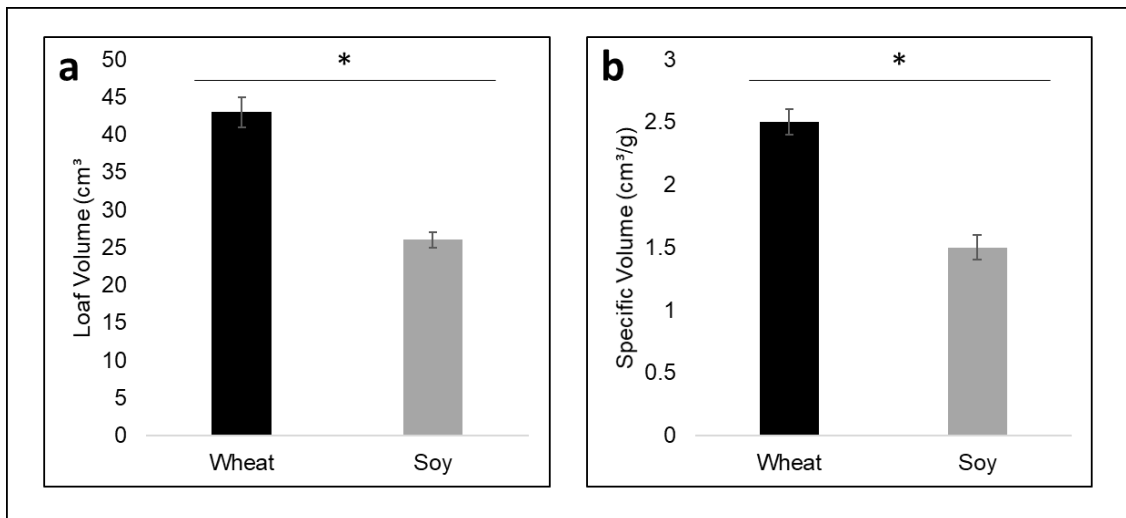
### *Statistical Analysis*

SAS version 9.4 (SAS Institute, Cary, NC) was used in all statistical analysis. Two-sample t-tests were used to determine differences between wheat and soy pretzel physicochemical properties. To determine differences in acceptability between athlete and non-athlete groups, Analysis of Variance (ANOVA) was performed with. Statistical significance was evaluated with an  $\alpha$  of 0.05. Average  $\pm$  standard deviation was used to report all values, apart from sensory evaluation data. Since sensory data was skewed median  $\pm$  interquartile range were used. Frequency style data was analyzed using a chi-square test. Chi square pairwise comparisons were used for post-hoc analysis, performed using a Bonferroni correction for multiple comparisons with a corrected significance level of 0.0083 (0.05 divided by 6 comparisons).

## Results and Discussion

### *Loaf volume*

The loaf volume of the wheat pretzels was significantly higher than that of the soy pretzels ( $p < 0.0001$ ), as was the specific volume ( $p < 0.0001$ ). Previous studies have shown reduced loaf volume in soy breads as opposed to wheat breads, possibly due to the soy proteins inhibiting the extensibility of the wheat gluten proteins<sup>23</sup>. The specific volume was reduced to an extent that was proportional with the reduction in loaf volume.



*Figure 1: The loaf volume (a) and specific volume (b) of wheat and soy pretzels. Asterisks represent statistical differences ( $p < 0.05$ ).*

### *Moisture, Freezable and Un-freezable Water*

During baking, the wheat pretzels lost  $13.90 \pm 0.52\%$  of their mass whereas the soy pretzels lost  $10.19 \pm 0.36\%$  of their mass, although the wheat pretzels contained less initial water. The difference in mass loss between samples was significant ( $p < 0.0001$ ). This could be due to the greater water binding capacity of soy flour as compared to wheat flour<sup>21</sup>. The ability for a dough to retain water can enhance the shelf life of that product, as moisture loss and

redistribution is one of the causes of bread staling<sup>34,35</sup>. Freshness of bread is one of the main qualities that contributes to acceptability and is related to moistness; therefore, moistness and reduced staling could contribute to acceptability<sup>36</sup>.

An average thermogram for wheat and soy pretzel crumb is displayed in Figure 2. Moisture content was determined by the percent weight loss at 180°C. The average moisture content of wheat pretzels was 39.1±1.0% and that of soy was 45.6±0.8%. The moisture content of the soy pretzels was statistically higher than that of the wheat (p=0.0009). This is likely due to the higher initial moisture content of the dough and the higher moisture retention after baking. Additionally, the derivative weight loss of the samples was analyzed to determine the temperature at which maximum weight loss occurred. These maxima occurred at 88.7±1.8°C and 105.1±3.1°C for wheat and soy pretzels respectively, and were significantly different (p=0.0014). The shift in maximum rate of moisture loss indicates a difference in the interaction of the soy dough ingredients with moisture. Soy proteins contain more hydrophilic amino acids than wheat proteins, which could lead to a greater interaction with water molecules<sup>23</sup>.

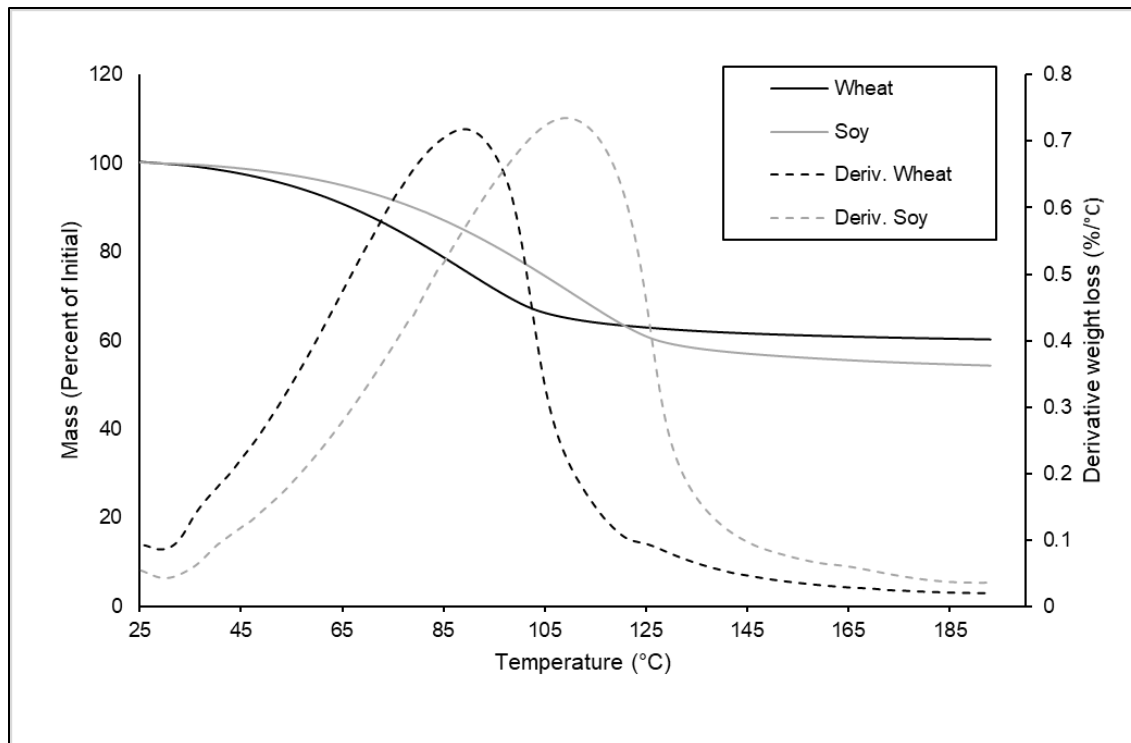


Figure 2: Example moisture desorption pattern of wheat and soy pretzels. Black and grey lines indicate wheat and soy samples, respectively. Solid lines show sample mass over increasing temperatures whereas dashed lines show the derivative weight loss.

The % freezable water of the wheat pretzels ( $20.3 \pm 0.2\%$ ) was lower in wheat pretzels than soy pretzels ( $27.7 \pm 0.3\%$ ),  $p < 0.0001$ . This is likely due to the increased initial water in the soy pretzels, and the reduced moisture loss on baking. The freezable water, however, did not differ between samples and was  $19.6 \pm 0.1\%$  in wheat pretzels and  $18.3 \pm 1.1\%$  in soy pretzels ( $p = 0.2044$ ). The increased freezable water is consistent with previous studies stating that the excess water added to the soy dough partitions to the freezable phase<sup>23</sup>. Increased freezable water in soy dough has been shown to reduce crystallization of amylopectin during storage and, therefore, attenuate bread staling<sup>23</sup>.

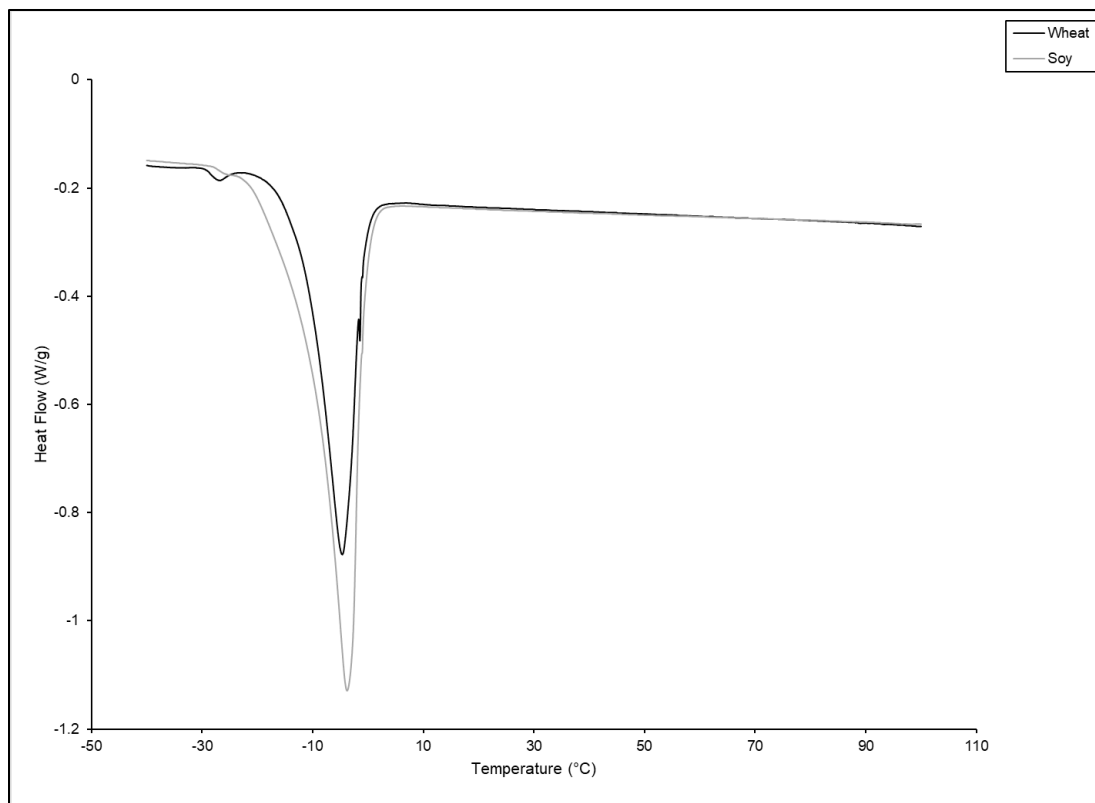


Figure 3: Differential scanning calorimetry of wheat and soy pretzels

### Texture Analysis

Three texture parameters including hardness, chewiness, and springiness were assessed in wheat and soy pretzels.

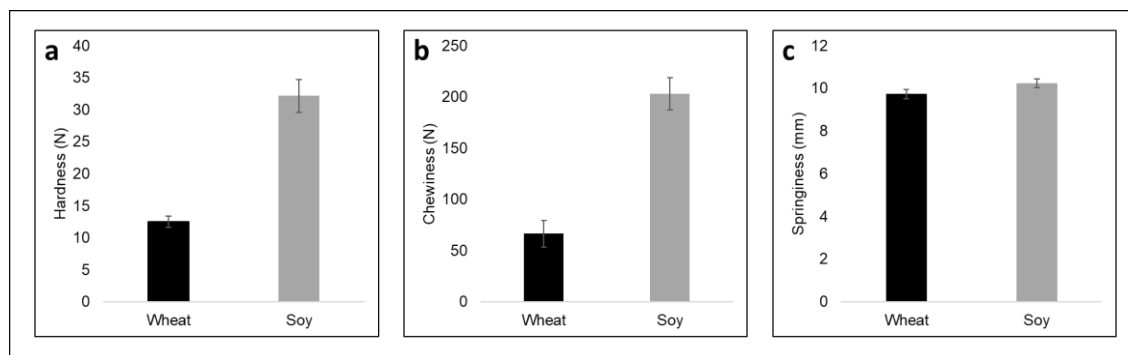


Figure 4: Texture profile analysis attributes of wheat and soy pretzels. (a=hardness, b=chewiness, c=springiness)

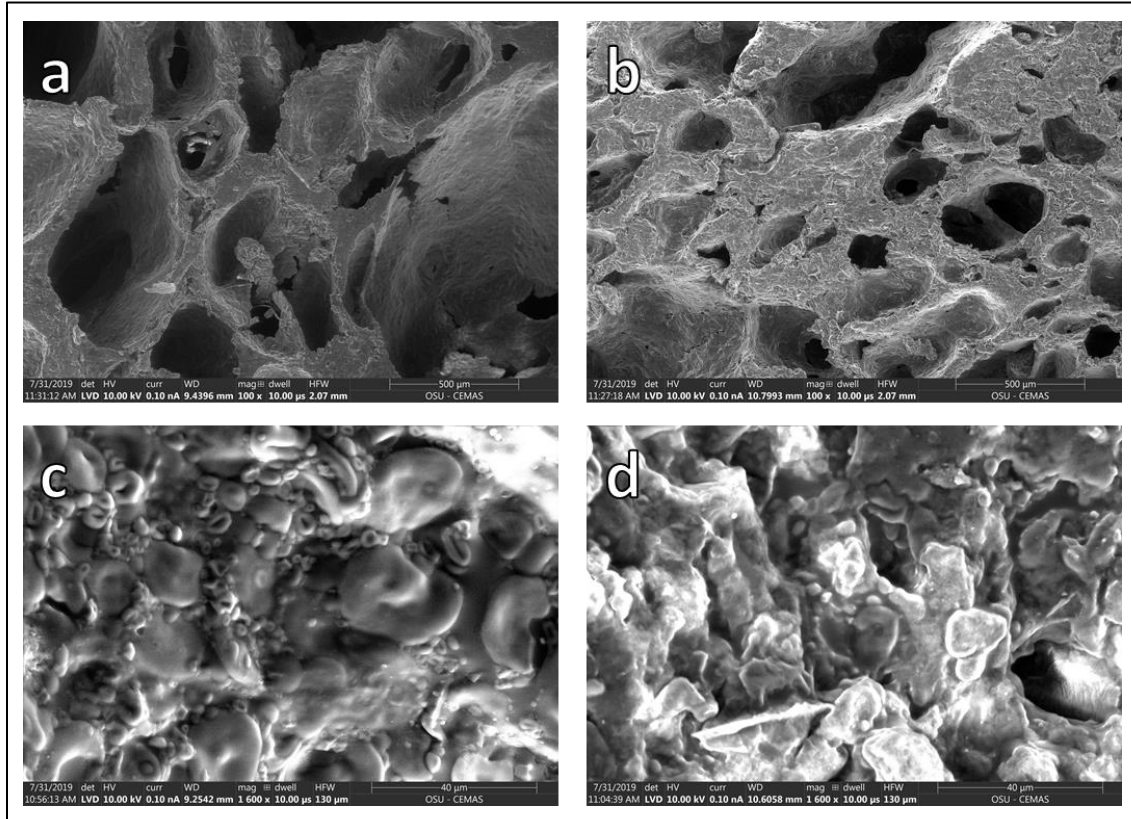
The texture profile of soy pretzels differed from the conventional wheat pretzel. Soy pretzels had a hardness that was 157% greater than that of the wheat pretzel ( $p < 0.0001$ ). The chewiness was also 205% greater ( $P < 0.0001$ ) and the springiness 5% greater ( $p = 0.005$ ) in the soy pretzel. Similar changes in texture have been observed in soy breads and pretzels previously, with increased firmness, chewiness, and springiness<sup>22,37</sup>. Hardness in bread products contributes to consumer perception of decreased “freshness”<sup>38,36</sup>; however, in soft pretzels this characteristic has not been defined. Chewy texture is a defining characteristic of soft pretzels, indicating that an increase in chewiness could be a desirable texture attribute. The springiness was significantly higher in the soy pretzel, but the difference was small, less than 1 mm of increased recovery.

#### *Scanning Electron Microscopy (SEM)*

Images were taken using SEM to visualize the internal microstructure of the baked pretzel crumb and are shown in Figure 5. Images at 100x magnification showed air cells that were larger in size in the wheat pretzel, indicating greater expansion during proofing and baking. The air cells in the soy pretzels were surrounded by thicker cell walls, which leads to greater springiness but a denser texture<sup>39</sup>. This increase in springiness is supported by TPA data, as thicker cell walls likely led to the firmer and springier texture. The lower dough extensibility could have inhibited the expansion of the air pockets in the soy dough<sup>40</sup>.

In the wheat 1600x magnification image, small, ungelatinized starch granules were visible. These were not visible to a great extent in the soy pretzels, either due to the soy and gluten proteins being in higher concentration and diluting the starch content or greater degree of starch gelatinization, possibility due to the higher initial moisture content. A dense surface without evidence of embedded starch molecules has been observed previously in soy breads<sup>40</sup>.

Another theory would be that the proteins surround the starch granules, so they are not visible in these images of the soy pretzels.



*Figure 5: SEM Images of pretzel crumb at 100x and 1600x magnification. (a=100x mag wheat, b=100x mag soy, c=1600x mag wheat, d=1600x mag soy)*

### *Isoflavone Quantification*

The wheat pretzels did not contain isoflavones at detectable levels. The total isoflavone concentration in the soy pretzels was found to be 0.18 mg total isoflavones /g baked pretzel on a wet weight basis. The isoflavone dose per pretzel piece was approximately 3.2 mg. The number of pretzel pieces could be adjusted based on the goal and requirements of different clinical trials. For exercise recovery, previous isoflavone doses have ranged from 21-22 mg per serving from

isolated soy protein<sup>9,10</sup>. Using a similar dose, an exercise study would need to use a dose of approximately 7 pretzel balls to reach this level.

Typical soy flour has an isoflavone concentration of approximately 1.20-3.81 mg/g and soymilk powder has a concentration of approximately 0.4-0.5mg/g<sup>18</sup>. It is estimated that the soy blend of 75% soy flour and 25% soymilk powder would contain 1.0-2.98mg/g. The soy blend used in this study contained only 0.76 mg/g. Considering moisture loss during baking, the final pretzel contains approximately 25.4% soy blend by mass. This would lead to an estimation of at least 0.20 mg/g isoflavones. The isoflavone concentration of the pretzel was slightly lower than expected, at 0.18 mg/g. Isoflavone concentration varies in soy flour based on soybean variety and environmental factors, which could have led to this discrepancy<sup>18</sup>. Additionally, there could have been loss of isoflavones or conversion to other forms that were not measured. Isoflavone levels should be monitored in future studies to ensure that dose remains constant.

### Sensory Evaluation

Sensory evaluation was performed with athletes in both the lab (n=100) and field (n=31) settings. In the lab, both athletes and non-athletes performed sensory testing, with non-athletes being divided by exercise frequency. The median exercise frequency of non-athletes was 3 days per week. Exercising greater than or equal to 3 days per week was classified as “active” and less than 3 days per week “less active”. In the field setting, only athletes performed sensory evaluation.

The distribution overall, appearance, aroma, flavor and texture liking values are shown in Table 3. The overall liking of soy pretzels for all panelists had a median of  $7 \pm 1$  out of 9, which corresponds to “like moderately” on the 9-point hedonic scale. The appearance, aroma, flavor,



and texture scores were  $8 \pm 2$ ,  $7 \pm 2$ ,  $7 \pm 2$ , and  $6 \pm 2$ , respectively. These attributes were ranked relatively high and correspond to a range of like slightly (6) and like very much (8).

There was no difference in overall liking between groups of active and less-active non-athletes or athletes ( $p=0.5416$ ). This indicates that liking was consistently high across the target populations of those who exercise and athletes. There was no difference in overall liking for athletes in the lab or field setting ( $p=0.6661$ ). No study at this point has evaluated how acceptability of foods may differ between athletes and non-athletes or by activity level<sup>41</sup>. However, athletes, compared to non-athletes, may have different priorities in terms of food selection, beyond acceptability or preference<sup>41</sup>. This study seems to suggest that acceptability did not differ between athletes and non-athletes with this product, but future work is needed to confirm that this is the case in a diverse group of athletes with a range of food products.

*Table 3: Sensory scores for overall, appearance, aroma, flavor, and texture acceptability by group and location. Data expressed as median  $\pm$  interquartile range due to data skew.*

Median Liking Score $\pm$ IQR	Lab			Field
	Infrequent-Exercisers (n=30)	Frequent-Exercisers (n=56)	Athletes (n=14)	Athletes (n=31)
Overall	$7.0 \pm 1$	$7.0 \pm 1.0$	$7.5 \pm 1.0$	$7.0 \pm 1.0$
Appearance	$8.0 \pm 1.0$	$8.0 \pm 1.0$	$8.0 \pm 1.0$	$8.0 \pm 1.0$
Aroma	$8.0 \pm 1.0$	$8.0 \pm 2.0$	$8.0 \pm 1.0$	$8.0 \pm 2.0$
Flavor	$7.0 \pm 2.0$	$7.0 \pm 1.5$	$7.0 \pm 1.0$	$8.0 \pm 1.0$
Texture	$7.0 \pm 3.0$	$6.0 \pm 2.5$	$7.0 \pm 3.0$	$7.0 \pm 1.0$

A penalty analysis was performed on each of the taste, aroma, texture, and appearance attributes tested. None of the attributes contributed to a mean drop of over 1 point in over 25% of the panelists, indicating that deficiencies in specific attributes did not influence acceptability in a large proportion of the population studied. Fifty-five percent of panelists did indicate that the pretzel was “too dry” but this led to a mean drop in score of 0.63 on the 9-point scale. Although

the mean drop is low, future product development could be targeted to reduce dryness. In order to meet the levels of isoflavones desired for exercise recovery (22-24 mg/serving), 7 pretzel pieces would need to be consumed. Panelists were asked if the serving size of 7 pieces was too small or too large using a JAR style question. Approximately 42% of panelists stated that this serving size was JAR, while 53% said it was too large. Therefore, adjusting the serving size to be smaller may be important for some consumers. This study did not assess the characteristics of the panelists that reported the above values; however, differences in lean body mass, satiety, or energy expenditure could have influenced their responses.

With high levels of soy in the pretzels, it was necessary to assess distinctive flavor characteristics of soybeans including bitterness and “beaniness”. In terms of bitterness, 86% of panelists indicated that the bitterness was JAR. In pretzels, bitterness is a common flavor attribute due to the extensive Maillard browning from the alkaline dip. Therefore, although there may have been bitter flavor present, it was still considered JAR. “Beaniness” was assessed in terms of aroma and flavor. The “beany” aroma had an average level of a  $2.8 \pm 1.9$  on a 10-point scale and the “beany” flavor,  $3.1 \pm 2.1$ . These values show that “beaniness” was present but at low levels.

Appropriateness of this product for a post-workout snack, specifically, was assessed. Overall, 52% of the panelists would consume this product after exercise. Within groups, in the lab setting 86% of athletes and 43% of active non-athletes stated that they would consume this product after exercise, along with 67% of athletes in the field setting. This indicates that the majority in the target populations would likely find this product appropriate for the stated application.

In conclusion, an acceptable soy-based soft pretzel was formulated to contain isoflavones at levels used previously in exercise studies (around 22.4 mg/serving), and a protein content of 28 grams per serving. The soy pretzels had a greater moisture content than the wheat pretzels, with the excess moisture partitioned in the freezable phase. Increased moisture could have implications in reducing bread staling rate. Future studies would be needed to evaluate staling and pretzel shelf life. The texture of the soy pretzels did differ from the wheat pretzels as shown by the TPA data and SEM images of air cell and cell wall sizes. Hardness has been shown to reduce acceptability in bread products previously, yet it did not affect sensory scores in this study. Although there were differences in the physicochemical properties of the soy pretzels from traditional wheat pretzels, the overall acceptability and appropriateness of the product was still high.

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