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The Effects of Incorporating Sprouted and Non-Sprouted Chickpea Flour in Pasta Products Upon Sensory Characteristics, Consumer Acceptability, In-Vivo Flow-Mediated Dilation, and In-Vitro TEAC Analysis

Joseph Adam Bruno

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Abstract:

Chickpeas (*Cicer arietinum L.*) is used in various products in the pasta industry. Amongst the numerous processing methods for chickpeas, sprouting increases chickpeas antioxidant content, nutrient bioavailability, and removes unwanted inhibitors.

Consuming antioxidants has shown to positively impact endothelial function, which is related to atherosclerosis prevention. **PURPOSE:** To explore the antioxidant potential of chickpeas, to explore how chickpea antioxidants are absorbed in the body, and to assess whether chickpea pasta is appealing to a consumer and sensory panel. **METHODS:** 108 healthy adults undertook a randomized sample consumer assessment of the likeability of 10 different pasta samples based on appearance, texture, flavor, and overall quality. The samples involved sprouted chickpea flour (SCF) and non-sprouted chickpea flour (NSCF) combined with semolina flour in a range of concentrations (0 %, 20 %, 40%), and all possible blends were evaluated for two different pasta shapes (fusilli and rigatoni); a total of 10 samples. Moreover, eight trained individuals participated in a descriptive analysis and assessed the pasta samples based on: chewiness, mushiness, overall strength of aftertaste, earthiness, pasta flavor, saltiness, sweetness, bitterness, strength of smell, and grittiness. Antioxidant potential was also assessed using Trolox Equivalent Antioxidant Capacity (TEAC) assay for seven samples (SCF and NSCF concentrations of 0 %, 20 %, 40%, and 100%). Moreover, healthy participants participated in a randomized, crossover, controlled meal study on two different days. Participants ingested 255.15 grams of pasta with 21.27 grams of butter. The experimental visit involved 40% sprouted chickpea flour and 60% semolina flour; the control visit involved 100% semolina flour. **RESULTS:** The consumer assessment results showed that the addition of sprouted chickpea to semolina

did not show significance in overall or willingness to purchase ($p > 0.05$). The descriptive analysis results showed that sprouted chickpea pasta had a significant increase on the pasta's earthiness, aftertaste, bitterness, grittiness, and a decrease effect on the pasta's pasta flavor. TEAC analysis showed the 100% SCF to have the highest antioxidant potential whereas unenriched semolina flour showed the lowest antioxidant potential ($p < 0.05$). Both 40% SCF and 40% NSCF had significantly greater antioxidant potential compared to unenriched semolina flour (USF) ($p < 0.05$). Flow Mediated dilation (FMD) was improved following the sprouted chickpea pasta ($10.28 \pm 1.19\%$) than the semolina pasta ($7.87 \pm 0.81\%$, $p < 0.05$). **CONCLUSION:** The results indicated that fractional substitution of semolina flour with NSCF or SCF produces a pasta that is appealing to consumers and improves *in-vitro* and *in-vivo* antioxidant potential.

MONTCLAIR STATE UNIVERSITY

The Effects of Incorporating Sprouted and Non-Sprouted Chickpea Flour in Pasta Products upon Sensory Characteristics, Consumer Acceptability, *In-Vivo* Flow-Mediated Dilation, and *In-Vitro* TEAC Analysis

By

Joseph Bruno

A Master's Thesis Submitted to the Faculty of

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In Partial Fulfillment of the Requirements

For the Degree of

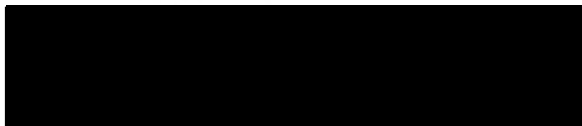
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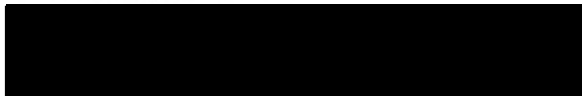
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1. Introduction

1.1 Introduction to the Origin of Sprouting in Human Nutrition

Legumes are naturally grown in nature with or without human intervention by going through the process of germination (Masood, Shah, & Zeb, 2014). The process of humans germinating seeds into an early sprouted state, specifically to be consumed during this stage of development, has been utilized since before there was recorded history (Kuo, Rozan, Lambein, Frias, & Vidal-Valverde, 2004; Lu & Cao, 2015; Mayer & Poljakoff-Mayber, 1982). The relationship between agriculture and sprouting was emphasized by authors such as Theophrastus of Eresus (Thanos, 2005) and many Roman authors (Antonkiewicz & Łabętowicz, 2016; Evenari, 1980). In many religions such as Christianity and Judaism, sprouts are established as a symbol of faith (Evenari, 1980). Historical evidence shows that ancient Egyptians added special containers containing sprouts as a symbol of resurrection after death (Evenari, 1980). Ancient documents dating back more than 5,000 years ago show that Chinese physicians prescribed sprouts for numerous health disorders (Lu & Cao, 2015). The interest of sprouting grew in the United States after World War II when a nutrition professor at Cornell identified the benefits of soybean sprouts in Vitamin C content (Loosli, 1973; Lu & Cao, 2015).

Legumes in their natural state are rich in protein content, which puts them in high demand in various cultures (Schutyser, Pelgrom, Van der Goot, & Boom, 2015).

Although legumes such as chickpeas (Rachwa-Rosiak, Nebesny, & Budryn, 2015), soy (C. Liu, Cheng, & Yang, 2017) and lentils (Shahwar, Bhat, Ansari, Chaudhary, & Aslam,

2017) are rich in protein, they contain small amounts of inhibitors such as trypsin inhibitors, protease inhibitors and other chemical substances (Q. Li, Wei, Yuan, Yang, & Ning, 2014; Masood et al., 2014). These inhibitors limit the bioavailability of their nutritional values, specifically their protein content (Q. Li et al., 2014; B. Liu et al., 2017; Masood et al., 2014). Thus, it is important that these inhibitors be removed in order to allow the body to effectively absorb the protein content within the digested legume. (Masood et al., 2014; Świeca & Baraniak, 2014; Zhang et al., 2015). Among the different processing methods, sprouting has shown to greatly increase the bioavailability of nutrients within the mentioned legumes (Gao, Yao, Zhu, & Ren, 2015; Masood et al., 2014; Świeca & Baraniak, 2014). Thus, sprouting is important because inhibitors are removed from nutrients within the legume, which increases their bioavailability (Gao et al., 2015; Gunashree, Kumar, Roobini, & Venkateswaran, 2014; Masood et al., 2014; Świeca, 2016). Therefore, evidence suggests legumes in early stages of sprouting are frequently more nutritious than legumes that have not yet begun the process of germination.

1.2 The Process of Sprouting (Germination)

It is well established that sprouting changes the chemical composition of seeds or legumes (Devi, Kushwaha, & Kumar, 2015; Frias, Miranda, Doblado, & Vidal-Valverde, 2005; Y.-C. Li et al., 2014; Masood et al., 2014; Villacrés et al., 2015). During germination, the seed comes out of the dormant stage (Kigel, Rosental, & Fait, 2015; Sangronis & Machado, 2007). As the seed transitions from the dormant stage, it needs

adequate humidity, temperature, and nutrients to grow (Villacrés et al., 2015). For the majority of the seeds, all the nutrients required for growth are stored within the seed (Kim et al., 2015; Sangronis & Machado, 2007). Environmental humidity enables the seed to access its stored nutrients (Masood et al., 2014). At this time the seed will germinate and grow into a mature plant (Panayidou & Apidianakis, 2017).

1.3 Antioxidant Properties of Sprouts

Multiple studies have found that germination increases the total antioxidant activity of legumes (Gharachorloo, Tarzi, & Baharinia, 2013; Kou et al., 2013; Masood et al., 2014; Mendoza-Sánchez et al., 2016; Peñas et al., 2015; Pulido, Bravo, & Saura-Calixto, 2000; Ramesh & Swami, 2016; Świeca & Baraniak, 2014). One common antioxidant found to increase during sprouting is known as ascorbic acid (Vitamin C). During germination, ascorbic acid more than doubles within various seeds (Masood et al., 2014). Peak nutrition quality of these seeds occurs within 120 hours of germination (Masood et al., 2014). This increase has been observed in a wide array of seeds and legumes including chickpeas, mung beans, kidney beans, lentils, cowpeas, and sunflower seeds (Devi et al., 2015; Dueñas, Martínez-Villaluenga, Limón, Peñas, & Frias, 2015; Masood et al., 2014; Pająk, Socha, Gałkowska, Rożnowski, & Fortuna, 2014; Peñas et al., 2015). Moreover, a large amount of literature confirms that soaking and cooking provide beneficial nutritional improvement (Aguilera, Esteban, Benítez, Mollá, & Martín-Cabrejas, 2009) with sprouting providing the best nutritional value (Masood et al.,

2014). Chickpeas differ between its cooked state, raw state, and sprouted state (Y. Xu, Thomas, & Bhardwaj, 2014).

Devi et al. (2015) concluded that the vitamin C content in cowpea (*Vigna unguiculata*) increased a range of 4-38 times in comparison to the original seed. Frias et al. (2005) also saw a similar increase in Vitamin C content in lupine seeds. In addition, the protein content of cowpeas was more bioavailable and the protein total content increased by 9-12% (Devi et al., 2015). This was due to the removal of the enzyme inhibitors present in the original dormant seed (Devi et al., 2015). These trypsin inhibitors decreased by 28-55%, allowing for more effective absorption of the protein content *in-vivo* (Devi et al., 2015). This was noted because there was an increase of 8-20% of *in-vitro* protein availability (Devi et al., 2015). However, vitamins such as vitamin C are heat sensitive and decrease during the heating process (Chen, Lang Jr, Graham, & Rizzutto, 1982; Devi et al., 2015; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008). Thus, it is important to consider when manufacturing products with cowpea sprouts that contain heat may affect its overall nutritional content (Devi et al., 2015).

Germination affects the bioavailability of vitamin E within white lupine seeds. Frias et al., (2005) found that germination in *Lupinus albus L.* (white lupine seeds) increases the vitamin E activity. The content of α -tocopherol increased in the seed and γ -tocopherol decreased (Frias et al., 2005). Although, the decrease in γ -tocopherol did not affect the δ -tocopherol activity resulting in increased vitamin E activity (Frias et al., 2005). Moreover, analysis of the antioxidant activity of the seed was performed by extraction of both hydrophilic and lipophilic extracts (Frias et al., 2005). The conclusion

was found that germination is a good treatment method to increase the antioxidant capacity, vitamin C, and vitamin E content in the seed (Frias et al., 2005).

Phenols react in the body as antioxidants (Cevallos-Casals & Cisneros-Zevallos, 2010; Gharachorloo et al., 2013; Khattak, Zeb, Bibi, Khalil, & Khattak, 2007; Randhir, Lin, & Shetty, 2004). These phenolic antioxidants provide physiological protection from free radicals within the human body (Blein et al., 2014; Cevallos-Casals & Cisneros-Zevallos, 2010; Kurfurstova et al., 2016; Pająk et al., 2014; Randhir et al., 2004; Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999). Cevallos-Casals & Cisneros-Zevallos, (2010) identified how plant sources can provide optimal changes in health by increasing the phenolic content. The process of germination increases the phenolic content and nutraceutical ability of mungbean, alfalfa, fava, fenugreek, mustard, wheat, sunflower seeds, soybean, and lentils (Cevallos-Casals & Cisneros-Zevallos, 2010). This increase in phenolics, in turn, increase the antioxidant function of the seed (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011; Randhir et al., 2004).

Randhir et al (2004) analyzed the total antioxidant property in fenugreek seeds after sprouting. It was concluded that antioxidant activity drastically increased after the seeds were sprouted (Randhir et al., 2004). This increase was caused by the enzyme guaiacol peroxidase (Randhir et al., 2004). This enzyme is required for the polymerization of the antioxidants otherwise known as phenolics within the seed to increase (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011; Randhir et al., 2004; Świeca, 2016). This enzyme is well known to become activated

during germination (Ghamsari, Keyhani, & Golkhoo, 2007; McCue & Shetty, 2004; Moccelini et al., 2010; Randhir et al., 2004; Thongsook & Barrett, 2005).

Świeca & Gawlik-Dziki, (2015) discussed how green peas and mung bean showed an increase of antioxidants during the first four days of sprouting. Wongsiri, Ohshima, & Duangmal, (2015) data showed that the antioxidant activity increased in Mung Bean after 24 hours of sprouting. The DPPH radical scavenging activity showed was 31.61% before germination and 62.84% after 24 h of germination (Wongsiri, Ohshima, & Duangmal, 2015). Moreover, oxidative damage has been associated with many chronic diseases such as cancer, cardiovascular disease, and diabetes (Luo & Xi, 2013; Scott, Rangaswamy, Wicker, & Izumi, 2014; Świeca, Baraniak, & Gawlik-Dziki, 2013; Świeca & Gawlik-Dziki, 2015; Woditschka et al., 2014). When sprouting, the legumes show a higher potential to reduce free radicals (Świeca & Gawlik-Dziki, 2015). Moreover, this increase also was associated with an increase *in-vitro* of the release of phenolics (Świeca & Gawlik-Dziki, 2015). Although, the quality of the sprouted seed depended greatly on the quality of the stored food in the seed (Świeca & Gawlik-Dziki, 2015).

Lintschinger et al., (1997) suggest that sprouting has an indication to reduce phytic acid content and reduces flatulence. This is due to the decrease in the presence of flatulence-causing oligosaccharides called stachyose and raffinose (Lintschinger et al., 1997). Although, one study analyzing Faba bean sprouts (*Vicia Faba L.*) found that germination time has a lot to do with the phytic acid content in the seed (Luo & Xi, 2013). The study found that phytic acid content decreases significantly after 72 hours of sprouting but then significantly increases after 120 hours of germination (Luo & Xi,

2013). In addition, the phenols extracted from the seed significantly increased within the first 24 hour time frame (Khattak, Zeb, Bibi, et al., 2007; Luo & Xi, 2013) (Luo & Xi, 2013). After this, the values of the phenol content decreased (Luo & Xi, 2013).

1.4 Minerals and Micronutrient Analysis of Germination

In addition to antioxidants, legumes also have many other compounds such as: enzyme inhibitors, lectins, and phytoestrogens (Boschin, Scigliuolo, Resta, & Arnoldi, 2014; Bouchenak & Lamri-Senhadji, 2013; Lima, Mota, Monteiro, & Ferreira, 2016; C. Liu et al., 2017; Mbithi, Van Camp, Rodriguez, & Huyghebaert, 2001; Urbana et al., 1995). All these bioactive compounds play a role in human digestion and metabolic rates (Bouchenak & Lamri-Senhadji, 2013). Many studies have analyzed the higher level of nutrients in sprouted seeds and legumes in comparison to the non-sprouted ones (Devi et al., 2015; Y.-C. Li et al., 2014; Masood et al., 2014; Mbithi et al., 2001; Nakitto, Muyonga, & Nakimbugwe, 2015; Ramesh & Swami, 2016; Sangronis & Machado, 2007). Y.-C. Li et al., (2014) analyzed the effect of germination on the nutritional content of peanut seeds. In the process of germination it was concluded that various amounts of nutrients changed when the peanut seed went through the process of germination (Kang et al., 2010; Y.-C. Li et al., 2014; K. H. Wang et al., 2005). The study concluded that the total phenolic content, mineral content (magnesium, calcium, and Iron), aspartic acid content, methionine content, proline content, folic acid content, and thiamine content increased substantially in peanuts after germination (Y.-C. Li et al., 2014). The peanuts

that were germinated also saw a decrease in the levels of fat found in the seed (Y.-C. Li et al., 2014).

Sprouting caused a significant increase in all measured nutritional values except in crude fat and carbohydrate in Cowpea legume (Devi et al., 2015). Although, there are many benefits noted it is important to consider that the cooking process affects the availability of some nutrients (Devi et al., 2015). Sangronis and Machado (2007) found similar results in an investigation of the effects of sprouting on the nutrient contents of black beans, white beans, and pigeon beans (Sangronis & Machado, 2007). *In-vitro* protein digestion replicated to resemble *in-vivo* digestion demonstrated that germination increased the digestibility of the protein content by 2–4% (Sangronis & Machado, 2007). Moreover, ascorbic acid digestibility increased by 33% for black beans, 208% for pigeon beans, and by 300% for white beans (Sangronis & Machado, 2007). In addition, the thiamine content increased by approximately 30% (Sangronis & Machado, 2007).

It is important to notice that germination is affected by the nutrient composition of each individual seed (Vidal-Valverde et al., 2002). Vidal-Valverde et al., (2002) analyzed the composition of inositol phosphates, vitamins B1, and B2 of beans, lentils, and peas. Moreover, Inositol from stored dry seeds come from alpha-galactosides. This study found that this substance was converted effectively to molecules of energy, such as glucose and fructose (Vidal-Valverde et al., 2002). For Vitamin B2 there was a significant increase but not for Vitamin B1 (Vidal-Valverde et al., 2002). All the results from this study were achieved after 6 days of germination (Vidal-Valverde et al., 2002).

After three days there seems to be a maximum increase of total starch content in lentils (Świeca et al., 2013). Moreover, total starch bioavailability seemed to be affected by the α -amylase inhibitors within the legume (Świeca et al., 2013). Although, when sprouting, there seems to be a modification of the starch content (Świeca et al., 2013). This is accomplished by the removal of the α -amylase inhibitors within the legume (Świeca et al., 2013). The conclusion of the Świeca et al., (2013) study suggests that germination is necessary to maximize the benefit of legume and seed consumption.

The expected glycemic index also increased reaching maximal levels after 5 days (Świeca & Gawlik-Dziki, 2015). The expected glycemic values were: 75.17 green peas, 83.18 lentils, and 89.87 for mung beans (Świeca & Gawlik-Dziki, 2015). Wongsiri, Ohshima, & Duangmal (2015) found that sprouting produces an increase in the availability of many amino acids within Mung Beans (Wongsiri et al., 2015). It was concluded that germination causes an increase in the number of amino acids; including phenylalanine (Wongsiri et al., 2015). Harini, Adilaxmamma, Mohan, Srilatha, & Raj, (2015) investigated the antihyperlipidemic effect of chickpea sprouts in mice. These studies suggest the importance of the germination process to the nutritional composition of legume seeds (Y.-C. Li et al., 2014; Sangronis & Machado, 2007; Wongsiri et al., 2015).

Phytoestrogens present in chickpeas have shown possible benefits to blood lipid levels (Gupta et al., 2016; Harini, Adilaxmamma, Mohan, Srilatha, & Raj, 2015). Phytoestrogens have been shown to improve the health of postmenopausal women (Gupta et al., 2016). Harini et al., (2015) used an ovariectomized mouse model to

compare phytoestrogens to a standard antihyperlipidemic agent (Harini et al., 2015). While the ovariectomy increased the total cholesterol and other lipid values, the dietary supplementation of sprouted chickpea normalized the lipid profile in the mice (Harini et al., 2015). It was concluded that sprouted chickpea present antihyperlipidemic ability by its ability to normalize the lipid levels in the mice (Harini et al., 2015). Although, there is yet to be an *in-vivo* study to analyze these specific effects of the phenolic content and Phytoestrogens.

1.5 Reduction of Inhibitors within Sprouted Seeds

In addition to the vitamin content increase, enzyme inhibitors within the seed are reduced within legumes (Bouchenak & Lamri-Senhadjji, 2013; Sangronis & Machado, 2007; Wongsiri et al., 2015). When the seeds are immersed in water, enzyme inhibitors are disabled, (Bouchenak & Lamri-Senhadjji, 2013; Masood et al., 2014; Wongsiri et al., 2015) which results in the initiation of the germination process (Masood et al., 2014). Once the inhibitor is disabled and detaches from the enzyme, the enzymes within the seed goes through complex biochemical changes (Masood et al., 2014). The same enzyme inhibitors that are disabled at the initiation of germination have been shown, in their active state, to inhibit the human body from absorbing proteins present within the legume (Emam, 2016; Khattak, Zeb, Khan, et al., 2007; Masood et al., 2014; Sangronis & Machado, 2007; Świeca et al., 2013).

A study involving Kidney beans (*Phaseolus vulgaris* var. Rose coco) found that there is a change in the Tannins within the seed (Mbithi et al., 2001). These Tannins

inhibit the human gastrointestinal tract from absorbing essential amino acids within the seed (Mbithi et al., 2001). Moreover, this analysis found that sprouting practically decreases the Tannin level of Kidney beans to an undetectable level (Mbithi et al., 2001). In addition, trypsin inhibitors within the bean also decrease by more than 70 percent (Mbithi et al., 2001).

Inhibitors within the non-sprouted seeds negatively affect the digestibility of the protein content (Devi et al., 2015; Frias et al., 2005; Mbithi et al., 2001). Mbithi et al., (2001), analyzed the removal of these inhibitors and found that it caused an improved *in-vitro* protein digestibility. The removal of these inhibitors causes a change in the extraction of calcium, iron, and zinc (Mbithi et al., 2001). There was an increase of calcium extractability by 55%, of iron by 54 %, and of zinc by 53% (Mbithi et al., 2001).

Sangronis & Machado, (2007) conducted a similar study with pigeon beans, black beans, and white beans. In their analysis, the trypsin inhibitors activity of pigeon beans upon sprouting decreased by 19.2%, in black beans by 25%, and in white beans by 52.5% (Sangronis & Machado, 2007). Moreover, the phytic acid was decreased by more than 40% in all the legumes analyzed (Sangronis & Machado, 2007). The tannins also decreased by 14.3% in pigeon beans, by 19% in black beans, and by 36.2% in white beans (Sangronis & Machado, 2007). The conclusion of both studies was that the data showed that sprouting presents an improvement in overall bioavailability of macronutrients as well as micronutrients (Mbithi et al., 2001; Sangronis & Machado, 2007).

1.6 Benefits of Pulse Legumes

There is sufficient evidence to state that legumes provide an improvement to overall health (Świeca et al., 2013). Currently, many nutritionist and dietitians are recommending pulses, also known as grain legumes (Wood, 2009). In addition to the nutritionist and dietitians, the American Diabetes Association (Polak, Phillips, & Campbell, 2015) and the American Heart Association (Stone et al., 2014) recommend pulses for better cardiovascular health and blood glucose control, as well as for a healthy source of protein and starch (Świeca et al., 2013; Wood, 2009). Nutritionist and dietitians have been trying to find a way to incorporate them into common food dishes that originally use grains such as durum wheat (Durazzo et al., 2013; Wood, 2009). It is possible in pulse legumes because they provide a color similar to grains such as durum wheat (D'Alessandro et al., 2016; Durazzo et al., 2013; Wood, 2009). This similarity would cause better adherence by the consumer and they would be more willing to use the pulse in replacement of the durum wheat (Durazzo et al., 2013; Sabanis, Makri, & Doxastakis, 2006; Wood, 2009).

Legumes are known for having a wide array of phytochemicals (Bouchenak & Lamri-Senhadji, 2013; Fares & Menga, 2014; Jensen et al., 2008). Consuming these phytochemicals have proven to have benefits towards a wide array of health conditions and ailments such as heart disease to diabetes (Bouchenak & Lamri-Senhadji, 2013). Researchers do not fully understand the exact role of phytochemicals and their benefits towards disease (Bouchenak & Lamri-Senhadji, 2013; S. Wang, Melnyk, Tsao, &

Marcone, 2011; B. Xu & Chang, 2009). More research is needed to determine the physiological mechanisms affected by these legumes which improve health and wellbeing (Bouchenak & Lamri-Senhadji, 2013). Doing so will give more clarity on the field of phytochemicals (Bouchenak & Lamri-Senhadji, 2013; Messina, 1999; B. Xu & Chang, 2009).

Chickpea legumes contain a wide array of vitamins and nutrients beneficial for the human body. In addition to this, the chickpea legume also contains high levels of polyphenolics (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011). These high level of polyphenolics give the seed a high level of antioxidant activity (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011; Bouchenak & Lamri-Senhadji, 2013; Khalil et al., 2007; Khattak, Zeb, Bibi, et al., 2007). These antioxidants are present in high quantities in the testa, the colored coat of the seed (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011). Observations of this study noted that soaking and steaming had an effect on the total phenolic content, total flavonoid content, and ferric reducing ability of the testa portion of the legume (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011). The authors recommended soaking for 22 hours and steaming for 1 hour to preserve the nutritional qualities of the legume (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011; Q. Li et al., 2014; Luo & Xi, 2013; Randhir et al., 2004).

1.7 History of Human Consumption of Chickpea (*Cicer arietinum L.*)

Many legumes are recognized as good sources of protein and other nutrients (Aguilera et al., 2009; Patil, Brennan, Mason, & Brennan, 2016; Schutyser et al., 2015). Chickpeas (*Cicer arietinum L.*) are calculated to have 25.3-28.9% protein content (Khattak, Zeb, Khan, et al., 2007). More recently chickpeas have been appreciated for their nutritional qualities beyond simply being a good source of protein (Boschin et al., 2014; Khattak, Zeb, Khan, et al., 2007; Patil et al., 2016). Legumes, such as chickpeas, are also a good source of carbohydrates and calories (Aguilera et al., 2009), and have been consumed in the Mediterranean diet (Gupta et al., 2016) and tropical region for centuries (Khattak, Zeb, Khan, et al., 2007).

In addition to the nutritional benefits, chickpeas offer a deep orange color and a taste that is not as “beany” as most other legumes (Fares & Menga, 2012a; Sabanis et al., 2006; Wood, 2009). These characteristics could potentially make it a suitable candidate to replace durum wheat in some food applications (Sabanis et al., 2006; Wood, 2009). One proposed means to increase the consumption of pulses is *via* the substitution of chickpea for wheat in the flour used to make pasta (Osorio-Díaz, Agama-Acevedo, Mendoza-Vinalay, Tovar, & Bello-Pérez, 2008; Wood, 2009). This substitution could potentially provide consumers with pasta products that present the nutritional benefits of pulses without detectable reductions in sensory quality (Flores-Silva, Berrios, Pan, Osorio-Díaz, & Bello-Pérez, 2014; Sabanis et al., 2006; Wood, 2009).

1.8 Chickpea (*Cicer arietinum L.*) Fortified Spaghetti

Chickpea pasta provides a gluten free option for individuals with gluten intolerance (Vijaykrishnaraj, Bharath Kumar, & Prabhasankar, 2014). Chickpea flour also provides non-digestible carbohydrates that are used in the body as fiber (Fares & Menga, 2014). There have been multiple studies trying to incorporate chickpea flour in common foods used in society (Fares & Menga, 2014; Jagannadham, Parimalavalli, Babu, & Rao, 2014; Ouazib, Dura, Zaidi, & Rosell, 2016; Padalino et al., 2015; Rizzello, Calasso, Campanella, De Angelis, & Gobetti, 2014; Sabanis et al., 2006; Wood, 2009). This is possible because chickpea flour has forming capacity and stability due to its protein content (Jagannadham et al., 2014).

Wood, (2009) studied consumer acceptability of chickpea fortified spaghetti. The study concluded that incorporating non-sprouted chickpea-fortified spaghetti was acceptable to consumers (Wood, 2009). In addition, incorporating chickpea to the pasta made with durum wheat did not substantially affect pasta quality (Sabanis et al., 2006; Wood, 2009). This was evidenced in the preparation of chickpea pasta in comparison to durum pasta (Sabanis et al., 2006; Wood, 2009). It was shown in the study that the chickpea pasta demonstrated less cooking loss, less stickiness, and retained firmness in comparison to the control spaghetti (Wood, 2009). In addition, it was noted that the chickpea pasta retained its firmness better after refrigeration than did the control (Wood, 2009). The chickpea pasta was found to be less sticky in comparison with the unfortified spaghetti (Wood, 2009). It was concluded in the study that increasing the pasta's protein content with the chickpea flour was associated with the decreased stickiness (Wood,

2009). On the contrary, the gluten content appeared to be associated with the pasta's firmness (Fares & Menga, 2014; Goñi & Valentín-Gamazo, 2003; Marti & Pagani, 2013; Osorio-Díaz et al., 2008; Wood, 2009).

Chickpea-fortified spaghetti is suitable to be made fresh, boiled or microwaved (Laleg, Barron, Santé-Lhoutellier, Walrand, & Micard, 2016; Sabanis et al., 2006; Wood, 2009). In addition, it is important to note that with the addition of chickpea flour to the pasta, the gluten content from the replaced wheat flour is also removed. This, in turn, affects the overall structure of the pasta. The study also verified that gluten content is associated with the pasta firmness (Sabanis et al., 2006; Wood, 2009). In addition, the pasta made of chickpea provided increased protein and amylose content, this is associated with the loss of the stickiness property of the pasta (Laleg et al., 2016; Wood, 2009). Arab et al. (2010), concluded that different processing methods of chickpea pasta also affects the appearance of the pasta (Arab, Helmy, & Bareh, 2010). By increasing the chickpea content, there was a decreased in the lightness and yellowness appearance of the pasta (Arab et al., 2010).

When mixing wheat flour with chickpea flour the texture of the dough changes. One study found that combining wheat flour and chickpea flour created a dough with higher strength and added elasticity (Sabanis et al., 2006). This study added more chickpea flour ranging from 5-20% and there was an improvement in both elasticity and strength of the lasagna dough (Sabanis et al., 2006). Although, when more than 30% was added to the flour the qualities deteriorated (Sabanis et al., 2006). The authors stated that deterioration could be due to the decrease in gluten content within the pasta (Sabanis et

al., 2006). Flores-Silva et al (2014) stated that gluten-free pasta made from chickpea had higher protein and fat in comparison to semolina flour pasta (Flores-Silva et al., 2014). In addition, there was a smaller amount of available starch to be absorbed in chickpea pasta in comparison with semolina pasta (Flores-Silva et al., 2014). This is associated with the glycemic index of the pasta (Flores-Silva et al., 2014). More research is required to fully understand the factors determining pasta sensory quality (Laleg et al., 2016; Sabanis et al., 2006; Wood, 2009).

1.9 Macronutrients of Chickpea (*Cicer arietinum L.*) Spaghetti

The preparation of pasta with chickpea flour causes the pasta to have increased protein, lipid, and dietary fiber content (Fares & Menga, 2012a; Osorio-Díaz et al., 2008; Sabanis et al., 2006; Wood, 2009) along with a decrease in the total carbohydrate of the pasta (Osorio-Díaz et al., 2008; Sun et al., 2015). The starch content was also modified with the addition of chickpea flour to the pasta (Osorio-Díaz et al., 2008; Sabanis et al., 2006). Most of the starch present in the chickpea fortified spaghetti has a large amount of fiber (Flores-Silva, Berrios, Pan, Osorio-Díaz, & Bello-Pérez, 2014); specifically 50% of the total starch in chickpea is resistant starch (Osorio-Díaz et al., 2008; Sabanis et al., 2006; Wood, 2009). Thus, most of the carbohydrates from the chickpea flour pasta is not absorbed.

When the chickpea content is increased in the pasta, the digestion improves (Osorio-Díaz et al., 2008). The digestion time is decreased with the increase of the added resistant starch content (Arab et al., 2010; Osorio-Díaz et al., 2008; Sabanis et al., 2006;

Wood, 2009). The glycemic index is lowered with the addition of chickpea flour (Osorio-Díaz et al., 2008). This means that chickpea flour starch content is slowly released into the bloodstream (Osorio-Díaz et al., 2008). Thus, chickpea pasta might present a dietetic alternative to the individuals who require low-calorie diets (Ferrara et al., 2006; Osorio-Díaz et al., 2008).

1.10 Sensory Properties of Chickpeas (*Cicer arietinum L.*)

Khalil et al. (2007) verified that sprouting has an effect on chickpeas protein solubility and sensory properties. This study found that the germination process involved in chickpeas had a dramatic increase in the moisture content of the seed, as well as the crude protein and crude fat contents (Khalil et al., 2007). It was also noted by the study that the nitrogen free extract saw a decrease when the chickpea seed was sprouted (Khalil et al., 2007). In addition, there was a significant increase of *in-vitro* protein digestibility when the chickpea seeds were sprouted (Khalil et al., 2007). On the contrary, during the first 24 hour period, there were overall better sensory scores for the chickpeas (Khalil et al., 2007).

Khalil et al. (2007) also found that in the first 24 hours of germination there was an improvement of overall sensory scores in comparison to cooked chickpeas. The maximum sensory score given was to the 24 hours sprouted sample (Khalil et al., 2007). Although, after the 24 hour sprouting period there was a significant decrease in scores given to the 96 hour sprouted samples. Moreover, the authors suggested that sprouts can

be considered as a new functional food (Khalil et al., 2007). Lastly, all the results from this study were in comparison to a 96 hour sprouting period (Khalil et al., 2007).

1.11 Nutritional Differences between Chickpea Flour and Wheat Flour

Legumes have also been used in the production of bread and studies have analyzed its stability in comparison with wheat based flour bread (Mohammed, Ahmed, & Senge, 2014; Ouazib et al., 2016; Rizzello et al., 2014). Mohammed, Ahmed, & Senge, (2014) analyzed incorporating various percentages of 10-30% of chickpea flour into wheat bread (Mohammed et al., 2014). It was found that increasing the chickpea flour concentration caused an increase in the viscosity parameters called the temperature of pasting (Mohammed et al., 2014). When incorporating 10% of chickpea flour there was a normal stickiness (Mohammed et al., 2014). Although, bread dough made with 20% and 30% chickpea flour caused a sticky dough surface (Mohammed et al., 2014). Another interesting quality was that increasing the chickpea flour content of the bread caused the bread to get darker (Mohammed et al., 2014). Arab et. Al (2010) demonstrated that chickpea flour provides an improved protein and fat content compared to wheat flour. In addition, the fiber and total carbohydrates are higher in wheat than in chickpea flour (Arab et al., 2010; Bouasla, Wójtowicz, & Zidoune, 2017; Mohammed et al., 2014). The various studies concluded that the incorporation of chickpea flour with wheat flour increases protein digestibility, mineral content, and amino acid scores (Arab et al., 2010; Bouasla et al., 2017; Laleg et al., 2016; Mohammed et al., 2014; Świeca et al., 2013).

1.12 Effect of Heat Treatment on Chickpea Flour Nutritional Quality

Fares & Menga, (2012) analyzed the effect of heat treatment on chickpea flour. It was examined how different toasting techniques affected the carbohydrates and antioxidants present in the chickpea flour (Fares & Menga, 2012b). When the chickpea fortified flour was heated, the antioxidant properties, resistant starch, and insoluble fiber increased (Fares & Menga, 2012b). The authors of this paper proposed that this is due to the high levels of free phenolic content in chickpea flour (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011; Fares & Menga, 2012a; Pająk et al., 2014; Randhir et al., 2004). Chickpea pasta contains higher concentration of free phenolic acid content compared to bound phenolic acid content whereas durum wheat contains a higher concentration of bound phenolic acid content in comparison to free phenolic content (Aharon, S., Hana, B., Liel, G., Ran, H., Yoram, K., Ilan, S., & Shmuel, 2011; Fares & Menga, 2012b). It was concluded that the total antioxidant capacity of the flour enriched with chickpea flour was dependent on the concentration of phenols in the system (Fares & Menga, 2012b).

1.13 In Summary

Germination is the only method of activating a dormant seed (Sangronis & Machado, 2007). The seed goes through the process of getting out of the dormant stage and prepares to become a plant organism (Sangronis & Machado, 2007). Research shows that sprouting provides a wide array of benefits for human consumption (Khalil et al., 2007). In addition to this, research shows that sprouting also removes inhibitors, which

allow for the better absorption of the vitamins and proteins present in the legumes of seeds (Wongsiri et al., 2015). With all this in consideration, the results show that partially substituting durum wheat flour with non-sprouted chickpea flour does not significantly affect the pasta's consumer acceptability. With this in consideration, it would be important to analyze the effects of incorporating chickpea flour and sprouted chickpea flour to pasta. Considering the aforementioned research, there seems to be a need for more investigation into the presence of antioxidants in sprouted chickpeas, how it is absorbed in the human body, and how to make food products that incorporate this ingredient. Therefore, the objectives of this study are: 1) Explore the antioxidant potential in chickpeas; 2) Explore how chickpea antioxidants are absorbed in the human body and 3) Assess whether chickpea pasta is acceptable to a consumer and sensory panel.

2. Manuscript I

Sprouted Chickpea and Non-sprouted Chickpea Pasta

based on

Consumer profiling, Sensory Evaluation,

and In-Vitro TEAC Antioxidant Analysis

2.1 Abstract

Chickpeas are commonly used in the food market to make pasta. Among the various processing methods, sprouting increases chickpeas antioxidant and nutrient bioavailability. However, it is unknown if sprouting chickpea pasta would be a viable product in the food market. **PURPOSE:** We hypothesized that pasta made partially from sprouted chickpeas would be an antioxidant rich and viable pasta to use for the general population. To determine the effect of incorporating Non-Sprouted Chickpea Flour (NSCF) and Sprouted Chickpea Flour (SCF) on the consumer acceptability, sensory quality, and antioxidant potential of the pasta. **Methods:** 108 lay adults participated in a randomized sample consumer assessment of the likeability of the appearance, texture, flavor, and overall quality of 10 different pasta samples. The samples incorporated sprouted chickpea flour (SCF) and non-sprouted chickpea flour (NSCF) with semolina flour in a range of concentrations (0 %, 20 %, 40%), and all possible flour blends were assessed for two different pasta shapes (fusilli and rigatoni). Additionally, descriptive analysis, utilizing eight trained panelists, assessed the different samples based on: chewiness, mushiness, overall strength of aftertaste, earthiness, pasta flavor, saltiness, sweetness, bitterness, strength of smell, and grittiness. Antioxidant potential was assessed for seven samples (SCF and NSCF composition of 0 %, 20 %, 40%, and 100%) in quadruplicate *via* the Trolox Equivalent Antioxidant Capacity (TEAC) assay. **Results:** The performed consumer assessment determined if the addition of sprouted chickpea to the pasta did not yield significant differences in overall or willingness to Purchase

($p > 0.05$). The descriptive analysis determined that sprouted chickpea pasta had a significant increase effect on the pasta's earthiness, aftertaste, bitterness, grittiness, and a decrease effect on pasta flavor. TEAC analysis showed the 100% SCF to have the highest antioxidant potential whereas unenriched semolina flour showed the lowest antioxidant potential. Both 40% SCF and 40% NSCF had significantly greater antioxidant potential compared to unenriched semolina flour (USF) ($p < 0.05$). **CONCLUSION:** Our results indicate that partial replacement of semolina flour with NSCF or SCF can improve antioxidant potential, and produce pasta that can appeal to consumers.

2.2 Introduction

Oxidative stress has been associated with numerous health concerns, such as cancer, coronary heart disease, cellular degradation from aging, and more (Blein et al., 2014; Kucinska et al., 2014; Kurfurstova et al., 2016). Antioxidant deficiency is a key factor in the effect of environmental toxicity on the carcinogenic process (Fuchs-Tarlovsky, 2013). This deficiency can be caused by genomic or environmental factors (Blein et al., 2014). Diets high in rich antioxidant sources such as legumes are associated with decreased prevalence of chronic diseases (Afshin, Micha, Khatibzadeh, & Mozaffarian, 2014; Castelló et al., 2014; Schröder, Marrugat, Vila, Covas, & Elosua, 2004).

Although legumes contain various vitamins and minerals that are beneficial to humans, they also contain trypsin and other inhibitors (Devi et al., 2015; Mbithi et al., 2001). These inhibitors limit the bioavailability of nutrients such as protein (Laleg et al.,

2017; Lima et al., 2016; Masood et al., 2014). Researchers have found that an effective method to improve the nutritional value of legumes is by sprouting (Masood et al., 2014). Sprouting the legumes allow for the inhibitors to be released, which increases the legumes' protein bioavailability (Masood et al., 2014). In addition, sprouting legumes have been found to increase the antioxidant potential of the legume (Chaparro-Hernández et al., 2015). Sprouting chickpeas has shown to increase the isoflavonoid, phenylalanine ammonia-lyase (PAL) activity and total antioxidant capacity (Guardado-Félix, Serna-Saldivar, Cuevas-Rodríguez, Jacobo-Velázquez, & Gutiérrez-Urbe, 2017).

In recent years, there has been a high demand from consumers to use chickpeas in pasta (Lee, Ng, Zivin, & Green, 2007). In comparison to 100% wheat flour, the incorporation of chickpea flour has been shown to improve the fat and protein content (Arab et al., 2010; Bouasla et al., 2017). Although the reduction of gluten decreases the structural stability of the pasta (Wood, 2009), the addition of chickpea flour to wheat flour has been shown to produce pasta of retained firmness without increasing stickiness (Bouasla et al., 2017; Wood, 2009). Even when chickpea concentration is increased to 100%, the internal structure of the pasta remains intact and viable (Bouasla et al., 2017).

The purpose of the current study was to determine the effects of adding both sprouted and non-sprouted chickpea flour to pasta on antioxidant potential, consumer experience, and sensory qualities. We hypothesized that fortifying pasta flour with chickpea flour would increase antioxidant potential and not significantly decrease consumer acceptability. Furthermore, we hypothesized that utilizing sprouted chickpea

flour would demonstrate greater antioxidant potential than non-sprouted chickpea flour and would retain its consumer acceptability.

2.3 Methods

Study population

All study procedures conformed to the provisions in the Declaration of Helsinki and were approved by the Institutional Review Board of Montclair State University. A total of 108 participants signed informed consent forms and participated in the consumer profiling protocol. In addition, a total of eight individuals signed informed consent forms in the sensory evaluation protocol.

Chickpea Sprouting Protocol

Goya chickpeas were used. The chickpeas were sprouted using a protocol previously described (Khattak, Zeb, Bibi, et al., 2007). Sprouting and food preparation took place in the food science laboratory at Montclair State University. In brief, the chickpeas were submerged in water for 18 hours. The non-absorbed water was discarded and the chickpeas were placed in a porous colander. The chickpeas were then rinsed with tap water for 2-3 minutes three times per day for 6 days. After the conclusion of days 2-7 the chickpeas were placed in a 0.56°C refrigerator overnight; to prevent bacterial growth (M. Kumar, Hora, Kostrzynska, Waites, & Warriner, 2006). At the conclusion of the last rinse on day 7, all the water was drained from the chickpeas. The Chickpeas were then placed into an Excalibur Food Dehydrator (8250 Ferguson Ave, Sacramento, CA, USA)

set at 48°C for 15 hours. At the conclusion of the incubation, the dehydrated sprouted chickpeas were then separated from the non-sprouted chickpeas. The sprouted chickpeas were then placed into a Vitamix Blender (8615 Usher Road, Cleveland, Ohio, USA) and ground into flour.

Semolina and Sprouted Chickpea Pasta Protocols

Five different flour compositions were prepared, representing incorporation of SCF and NSCF into semolina flour at a range of concentrations (0 %, 20 %, and 40%). Each of the flour compositions were prepared into two different shapes (fusilli and rigatoni), producing 10 samples in total. The “control” semolina flour was Bob's Red Mill Semolina Flour. The NSCF was prepared by grinding the Goya chickpeas in a Vitamix blender. The production of dough from flour was consistent for all sample types, accomplished by combining 400g of the flour mixture with 118mL of water. The flour mixture was mixed with water until it formed a solid dough. This dough was then kneaded and split into two sections, each individually wrapped in plastic wrap and left to sit at room temperature for 10 minutes. The dough was then formed into smaller balls and placed into a Kitchen Aid Gourmet Pasta Press attachment of a Kitchen Aid machine (553 Benson Road, Benton Harbor, MI 49022) and used to make fusilli and rigatoni pasta shapes. The pasta was left in the refrigerator at 0.56°C for 24 hours. The pasta then was split into 3 cups and placed in 710mL of boiling water for 5 minutes.

Consumer Assessment

Participants for the consumer assessment were excluded if they had any food allergies relevant to wheat, gluten, chickpea, or pasta sauce. Consumers were asked to evaluate the samples based on appearance, texture, flavor, overall taste, and willingness to purchase. Random numbers were assigned to each of the ten samples. Participants were given four different samples in counterbalanced orders.

Descriptive Analysis

Participants were recruited and were excluded if they had any food allergies relevant to wheat, gluten, chickpea, or pasta sauce. The participants were calibrated based on standards known in the food industry for sweetness, saltiness, earthiness, bitterness, and pasta flavor. Seven samples were coded and given at each visit in the same order for each participant for a total of three visits. The participants were then asked to evaluate the samples based on chewiness, mushiness, the overall strength of aftertaste, earthiness, pasta flavor, saltiness, sweetness, bitterness, the strength of smell, and grittiness.

Extraction and Evaporation Protocol:

Extraction was prepared as described in (Bonoli, Verardo, Marconi, & Caboni, 2004). Specifically, five grams of various NSCF and SCF flour concentrations (0 %, 20 %, 40%, and 100%) were prepared and combined with a 40 ml of a 4:1 acetone/water solution. Caputo Semola Di Grano Duro Rimacinata Semolina Flour was used to complete the remaining percentage of each sample. The solution was sonicated in an Ultrasonic Bath for 10 minutes and centrifuged for 10 minutes. The supernatant was

collected and the extraction was performed twice in each flour concentration. The evaporation was conducted at 40°C at approximately 307 mBar. The pressure was decreased until all possible acetone was removed. The remaining phenolic sample was combined with 5 ml of 99.7/0.3 water/formic acid solution. The final volume was standardized with deionized water to have a standard volume of 30 mL per sample.

A Trolox Equivalent Antioxidant Capacity (TEAC) assay

TEAC was evaluated as described in Brand-Williams, Cuvelier, & Berset (1995). Specifically, 25 mL DPPH sample was prepared in a 4:1 methanol/ H₂O solution at a 101.445µM concentration. A 99.7:0.3 water/formic acid solution zero solution was prepared as a standard. Various concentrations of 0-5mM Trolox solutions were prepared in a 1:1 acetone-water solution for the development of a standard curve. 10 µL of each sample including Trolox, zero solution, and flour supernatant was then added to the VersaMax ELISA Microplate Reader (1311 Orleans Drive Sunnyvale, CA 94089 USA), and readings were taken at 517 nm after 30 minutes.

Statistical Analysis

Consumer profiling was assessed using two-way ANOVA ($\alpha = 0.05$). A variety of comparisons were achieved using Tukey's HSD multiple comparison test ($\alpha = 0.05$). Modeling of association between the various qualities of the consumer profiling and sensory aspects of the samples were utilized. Models were built utilizing stepwise regression at $\alpha = 0.05$. All data analyses were performed using SPS software for Windows. Data is presented as mean \pm SD.

2.4 Results and Discussion

Consumer Assessment

The results of the consumer assessments of the pasta samples are shown in Table 1. For the majority of comparisons, there were no significant differences found. The only significant difference between samples for the trait of appearance was between 40% SCF/ Rigatoni and 20% NSCF/ Rigatoni, for which the 20% NSCF/ Rigatoni sample performed significantly worse ($p < 0.05$). In the case of texture, the first significance found was 100% Semolina/ Rigatoni and 40% NSCF/ Rigatoni, of which 100% Semolina/ Rigatoni had a worse texture ($p < 0.05$). The second significance was found between 20% NSCF/ Rigatoni and 40% NSCF/ Rigatoni, of which 20% NSCF had worse texture ($p < 0.05$). In the case of flavor, the only significance found was between 100% Semolina/ Fusilli and 40% SCF/ Fusilli, of which 40% SCF Fusilli had a lower result ($p < 0.05$). In the case of overall, there was only one significance between 100% Semolina/Fusilli and 20% NSCF/ Rigatoni of which 20% NSCF/ Rigatoni had a lower overall rating ($p < 0.05$). In the case of willingness to purchase, there was no significance between the results ($p > 0.05$). These result concurred with previous studies that found that incorporating chickpea flour to semolina did not substantially affect pasta quality (Sabanis et al., 2006; Wood, 2009).

In our development of multivariate predictive models, we found that texture scores were significantly positively associated with increasing concentrations of SCF ($p = 0.016$), but was not significantly associated with concentrations of NSCF. Neither SCF

concentration nor NSCF concentration were significantly associated with ratings of texture. SCF concentration was a significant negative predictor of ($p = 0.021$) of the ratings of flavor, while NSCF concentration was not. Neither SCF concentration nor NSCF concentration were significantly associated with ratings of overall quality or willingness to purchase.

Our models found the factor of pasta flavor to be significantly associated with assessments of texture ($p = 0.001$) and overall (0.021). In both cases, the rigatoni shape was associated with decreases in assessed values. There was a negative response to the rigatoni vs fusilli NSCF, both 20% ($p < 0.05$) and 40% ($p < 0.05$). Overall, incorporating sprouted chickpea flour in fusilli pasta seemed to have better consumer acceptability in comparison to rigatoni. This is possibly due to the thickness of the pasta, of which rigatoni is thicker compared to fusilli. No factors showed significant differences for the willingness to purchase trait. Our findings suggest some reduction in flavor quality associated with incorporation of SCF, but not significant reductions in overall quality or a consumer's willingness to purchase the product.

With predictive models using the data from table 1, it was determined that for increasing concentrations of SCF ($p < 0.05$) and NSCF ($p < 0.05$) chewiness decreased significantly. Chewiness at high values is perceived as a negative attribute. Predictive models also determined that if the parameters of shape, NSCF ($p < 0.05$) and SCF ($p < 0.05$) concentrations were known that 28% of the chewiness value could be calculated. The models also determined that rigatoni was less chewy and increases in NSCF ($p < 0.05$) and SCF ($p < 0.05$) were associated with a decrease in chewiness. Mushiness was

showed to be influenced by the concentrations of both SCF ($p < 0.05$) and NSCF ($p < 0.05$).

Descriptive Analysis

The results of the consumer assessments of the pasta samples are shown in Tables 2A and 2B. The significant difference between samples for the trait of chewiness was seen between various samples, first between 100% Semolina/ Fusilli and 40% NSCF/ Fusilli; of which 40 % NSCF/ Fusilli was less chewy ($p < 0.05$). The second was between 100% Semolina/ Rigatoni and 40% SCF/ Rigatoni, of which 40% SCF/ Rigatoni was less chewy ($p < 0.05$). The third was between 100% Semolina/ Fusilli and 40% SCF/ Fusilli, of which 40% SCF/ Fusilli was less chewy ($p < 0.05$). The fourth was between 40% NSCF/ Fusilli and 20% NSCF/ Fusilli, of which 40% NSCF/ Fusilli was less chewy ($p < 0.05$). In the case of mushiness, only one significant difference was found between 100% Semolina/ Rigatoni and 40% SCF/ Rigatoni, of which 100% Semolina/ Rigatoni was less mushy ($p < 0.05$). In the case of strength of aftertaste, the first significant difference was between 100% Semolina/ Fusilli and 40% SCF/ Fusilli, of which 40% SCF/ Fusilli had a stronger aftertaste ($p < 0.05$). The second significant difference was between 100% Semolina/ Rigatoni and 40 % SCF/ Rigatoni, of which 40 % SCF/ Rigatoni had a stronger aftertaste ($p < 0.05$). The third significant difference was between 100% Semolina/ Rigatoni and 20% SCF/ Rigatoni, of which 20% SCF/ Rigatoni had a stronger aftertaste ($p < 0.05$). In the case of earthiness, the first significant difference was between 100% Semolina/ Fusilli and 40% SCF/ Fusilli, of which 40% SCF/ Fusilli was earthier ($p < 0.05$). The second significant difference was between 100% Semolina/ Rigatoni and

40% SCF/ Rigatoni, of which 40% SCF/ Rigatoni was earthier ($p < 0.05$). The third significant difference was between 100% Semolina/ Rigatoni and 20% SCF/ Rigatoni, of which 20% SCF/ Rigatoni was earthier ($p < 0.05$). The increase in earthiness was considered to be a negative attribute.

In the case of pasta flavor, the first significant difference was between 100% Semolina/ Fusilli and 40% SCF/ Fusilli, of which 100% Semolina/ Fusilli had a stronger pasta flavor ($p < 0.05$). The third significance was between 100% Semolina/ Fusilli and 20% SCF/ Fusilli, of which 100% Semolina/ Fusilli had a stronger pasta flavor ($p < 0.05$). The fourth significance was between 100% Semolina/ Rigatoni and 40% NSCF/ Rigatoni, of which 100% Semolina/ Rigatoni had a stronger pasta flavor ($p < 0.05$). The fifth significant difference was between 100% Semolina/ Rigatoni and 40% SCF/ Rigatoni, of which 100% Semolina/ Rigatoni had a stronger pasta flavor ($p < 0.05$). The sixth significance was between 100% Semolina/ Rigatoni and 20% NSCF/ Rigatoni, of which 100% Semolina/ Rigatoni had a stronger pasta flavor ($p < 0.05$). The seventh significant difference was between 100% Semolina/ Rigatoni and 20% SCF/ Rigatoni, of which 100% Semolina/ Rigatoni had a stronger pasta flavor ($p < 0.05$).

For bitterness, all samples were significantly different in comparison to 40% SCF/ Rigatoni, of which 40% SCF/ Rigatoni was most bitter ($p < 0.05$). In the case of grittiness, all samples were significantly different in comparison to 40% NSCF/ Rigatoni and 40% SCF/ Rigatoni, of which both presented with the most grittiness ($p < 0.05$). In the analysis of texture, the first significance found was 100% Semolina/ Rigatoni and 40% NSCF/ Rigatoni of which 100% Semolina/ Rigatoni had a worse texture ($p < 0.05$).

The second significance found was between 20% NSCF/ Rigatoni and 40% NSCF/ Rigatoni of which 20% NSCF had worse texture ($p < 0.05$). In the case of saltiness, sweetness, and strength of smell there were no significant differences as expected because neither pasta was significantly different in terms of saltiness, sweetness, or strength of smell ($p > 0.05$).

Predictive models using that data from tables 2A and 2B confirmed that shape was not determined to have a significant effect on mushiness ($p < 0.05$). Aftertaste was significantly positively associated with increases in SCF ($p < 0.05$) and NSCF ($p < 0.05$) concentrations. Although, NSCF, as incorporated to the pasta, showed less of the aftertaste in comparison to SCF. The shape did not influence aftertaste. Earthiness was negatively affected by SCF ($p < 0.05$) and NSCF ($p < 0.05$). SCF, NSCF, and Shape showed to increase earthiness. Rigatoni had more earthiness than fusilli ($p < 0.05$). Pasta flavor was significantly negatively associated with increases in both SCF ($p < 0.05$) and NSCF ($p < 0.05$). Saltiness and sweetness were not affected by the incorporation of SCF and NSCF and shape. Bitterness was influenced by the addition of SCF ($p < 0.05$). NSCF and shape did not influence bitterness of pasta. The strength of smell was positively influenced by SCF ($p < 0.05$). NSCF and shape did not affect the strength of smell. Grittiness was affected by SCF and NSCF. Both NSCF and SCF showed to influence grittiness of pasta ($p < 0.05$). From these findings, it can be concluded that NSCF and SCF affect the texture, flavor, and appearance of the pasta. Qualities such as earthiness and strength of aftertaste were as expected to be higher in SCF due to the characteristic of the flavor of sprouted legumes (Khalil et al., 2007; Khattak, Zeb, Bibi, et al., 2007).

TEAC Analysis

Antioxidant analysis according to TEAC showed that 100% SCF had the highest antioxidant potential, with a TEAC value significantly greater than all other assessed samples ($p < 0.05$). 100% NSCF also had high antioxidant values, but not significantly higher than those of the 40% SCF sample and the 40% NSCF samples. Between the samples evaluated by sensory analysis, the 40% SCF and 40% NSCF samples had the highest TEAC values, both of which were significantly greater than the TEAC value of the 100% semolina sample. Our predictive model showed chickpea flour concentration to be significantly positive associated with assessed antioxidant potential ($p < 0.05$).

Conclusion

Our data indicates that incorporation of SCF may influence individual quality parameters of pasta, but up to 40% incorporation of SCF is not associated with a significant reduction in overall acceptability of the sample for the consumer. Certain characteristics seem to be influenced in SCF flour such as bitterness, the strength of smell, chewiness, aftertaste, pasta flavor, and strength of smell. SCF-rich pasta flour was also shown to have significantly greater antioxidant potential compared to 100% semolina flour. Our results indicate that incorporating SCF into pasta products may be an effective and viable mechanism for the delivery of antioxidant-rich foods to consumers.

2.5 Tables and Figures

Table 1 Consumer Assessment of Pasta Samples (mean±SD)

Sample	Appearance	Texture	Flavor	Overall	Willingness to Purchase
100% Semolina	Fusilli 4.38±1.50 AB	4.67±1.5 7 B	5.05±1.3 3 B	4.86±1.3 0 B	3.48±1.48 A
	Rigatoni 4.29±1.37 AB	3.59±1.4 5 A	4.27±1.4 7 AB	4.10±1.5 3 AB	2.90±1.41 A
40% NSCF	Fusilli 3.93±1.42 A	4.36±1.6 1 AB	4.36±1.5 2 AB	4.51±1.5 9 AB	3.14±1.50 A
	Rigatoni 4.58±1.26 AB	4.67±1.3 7 B	4.60±1.5 4 AB	4.67±1.3 7 AB	3.31±1.36 A
40% SCF	Fusilli 4.63±1.48 AB	4.41±1.2 5 AB	3.90±1.4 1 A	4.25±1.3 2 AB	2.75±1.32 A
	Rigatoni 4.63±1.24 B	4.29±1.4 4 AB	4.29±1.4 2 AB	4.40±1.3 5 AB	3.05±1.51 A
20% NSCF	Fusilli 4.27±1.53 AB	4.70±1.6 3 B	4.57±1.2 7 AB	4.48±1.3 4 AB	3.18±1.17 A
	Rigatoni 3.90±1.39 A	3.60±1.6 3 A	4.19±1.6 2 AB	3.71±1.5 2 A	2.50±1.44 A
20% SCF	Fusilli 4.36±1.42 AB	4.55±1.3 7 AB	4.56±1.3 9 AB	4.63±1.1 6 AB	3.19±1.48 A
	Rigatoni 4.44±1.35 AB	4.02±1.5 2 AB	4.12±1.3 8 AB	4.23±1.3 3 AB	3.00±1.46 A

Descriptive characteristics of pasta samples according to flour composition (mean ± SD).

Values calculated based on a consumer population of 108 individuals. Those followed by the same letter within a column (for each sample) were not significantly different from

one another ($p \leq 0.05$) according to ANOVA and means separation with Tukey's

Studentized Range *via* SAS software. Index; NSCF Non-Sprouted Chickpea Flour; SCF

Sprouted Chickpea Flour.

Table 2A Descriptive Analysis of Pasta Samples (mean±SD)

Sample		Chewiness	Mushiness	Strength of Aftertaste	Earthiness	Pasta Flavor
100% Semolina	Fusilli	0.62±0.20 CD	0.24±0.25 AB	0.19±0.13 A	0.10±0.07 A	0.61±0.23 BC
	Rigatoni	0.78±0.14 D	0.13±0.17 A	0.22±0.15 A	0.25±0.22 AB	0.67± 0.20 C
40% NSCF	Fusilli	0.26±0.19 A	0.48±0.25 B	0.29±0.25 AB	0.30± 0.18 ABC	0.38± 0.27 AB
	Rigatoni	0.49±0.26 ABC	0.41±0.25 AB	0.40±0.28 ABC	0.44±0.24 BCD	0.32±0.24 A
40% SCF	Fusilli	0.35±0.20 AB	0.43±0.28 B	0.57±0.26 BC	0.57± 0.25 CD	0.22± 0.19 A
	Rigatoni	0.42±0.26 ABC	0.47±0.32 B	0.63±0.33 C	0.60±0.28 D	0.19±0.20 A
20% NSCF	Fusilli	0.53±0.22 BCD	0.28±0.23 AB	0.37±0.21 ABC	0.33±0.25 ABCD	0.45±0.21 ABC
	Rigatoni	0.66±0.22 CD	0.31±0.25 AB	0.37±0.26 ABC	0.46±0.24 BCD	0.31±0.22 A
20% SCF	Fusilli	0.45±0.28 ABC	0.29±0.24 AB	0.42±0.27 ABC	0.37± 0.26 ABCD	0.32±0.24 A
	Rigatoni	0.54±0.27 BCD	0.28±0.30 AB	0.56±0.27 BC	0.57±0.26 D	0.34±0.25 A

Sensory characteristics of pasta samples according to flour composition. Values calculated based on 8 observations by trained panelists. Those followed by the same letter within a column (for each sample) were not significantly different from one another ($p \leq 0.05$) according to ANOVA and means separation with Tukey's Studentized Range *via* SAS software. Index; NSCF Non-Sprouted Chickpea Flour; SCF Sprouted Chickpea Flour.

Table 2B Descriptive Analysis of Pasta Samples (mean±SD)

Sample		Saltiness	Sweetness	Bitterness	Strength of Smell	Grittiness
100% Semolina	Fusilli	0.06±0.05 A	0.25±0.19 A	0.06±0.04 A	0.15±0.12 A	0.08±0.08 A
	Rigatoni	0.15±0.15 A	0.24±0.19 A	0.13±0.15 A	0.16±0.14 A	0.12±0.12 A
40% NSCF	Fusilli	0.14±0.15 A	0.20±0.19 A	0.17±0.18 AB	0.24±0.23 A	0.19±0.2 AB
	Rigatoni	0.08±0.07 A	0.25±0.17 A	0.22±0.18 AB	0.27±0.26 A	0.38±0.23 C
40% SCF	Fusilli	0.13±0.09 A	0.19±0.17 A	0.28±0.25 AB	0.31±0.27 A	0.26±0.26 AB
	Rigatoni	0.11±0.12 A	0.18±0.13 A	0.38±0.31 C	0.35±0.29 A	0.41±0.29 C
20% NSCF	Fusilli	0.09±0.08 A	0.31±0.2 A	0.12±0.16 A	0.23±0.23 A	0.21±0.21 AB
	Rigatoni	0.11±0.12 A	0.23±0.15 A	0.25±0.2 AB	0.32±0.28 A	0.26±0.19 AB
20% SCF	Fusilli	0.11±0.09 A	0.23±0.2 A	0.28±0.24 AB	0.34±0.28 A	0.21±0.19 AB
	Rigatoni	0.18±0.2 A	0.17±0.12 A	0.27±0.27 AB	0.35±0.28 A	0.30±0.23 AB

Sensory characteristics of pasta samples according to flour composition (mean ± SD).

Values calculated based on 8 observations by trained panelists. Those followed by the same letter within a column (for each sample) were not significantly different from one another ($p \leq 0.05$) according to ANOVA and means separation with Tukey's Studentized Range *via* SAS software. Index; NSCF Non-Sprouted Chickpea Flour; SCF Sprouted Chickpea Flour.

Table 3 TEAC Analysis of Antioxidant Potential

Sample	TEAC
100% Semolina	0.186 ± 0.002 D
20 % NSCF	0.194 ± 0.004 BCD
40% NSCF	0.198 ± 0.001 BC
100% NSCF	0.206 ± 0.004 B
20% SCF	0.192 ± 0.002 CD
40% SCF	0.203 ± 0.004 BC
100% SCF	0.236 ± 0.003 A

Descriptive characteristics of pasta samples according to flour composition (mean ± SD). Values calculated based on Trolox equations. Those followed by the same letter within a column (for each sample) were not significantly different from one another ($p \leq 0.05$) according to ANOVA and means separation with Tukey's Studentized Range *via* SAS software. Index; NSCF Non-Sprouted Chickpea Flour; SCF Sprouted Chickpea Flour.

3. Manuscript II

The Effect of Incorporating Sprouted Chickpea Flour in Pasta Products

Analyzed by

In-Vivo flow-mediated dilation

3.1 Abstract:

Antioxidant consumption has been shown to positively impact endothelial function. Endothelial dysfunction is a precursor to atherosclerosis. However, it is unknown whether dietary consumption of antioxidants in the form of sprouted chickpea flour will impact endothelial function. We hypothesize that brachial artery flow-mediated dilation (FMD), a test of endothelial function, would be increased after consuming pasta made with 40% sprouted chickpea flour vs. pasta made with 100% semolina flour. **PURPOSE:** To determine if incorporating sprouted chickpea flour to pasta will impact the results of *in-vivo* flow-mediated dilation. **METHODS:** Healthy participants underwent a randomized, crossover, controlled meal study on two separate days. Participants consumed 255.15 grams of pasta with 21.27 grams of butter. The experimental visit pasta composition was 40% sprouted chickpea flour and 60% semolina flour, control visit was 100% semolina flour. Two hours of digestion time was allowed before each FMD test. **RESULTS:** Twenty-nine (6 men, 23 women, age 25.41 ± 1.51 yrs, height 164.66 ± 1.42 cm, weight 66.43 ± 2.29 kg, body fat $25 \pm 2\%$) participated in the study. FMD was greater following the sprouted chickpea pasta ($10.28 \pm 1.19\%$) than the semolina pasta ($7.87 \pm 0.81\%$, $p < 0.05$). **CONCLUSION:** Partial replacement of semolina flour with sprouted chickpea flour acutely improves vascular function which is possibly due to its antioxidant potential.

3.2 Introduction

In America, cardiovascular disease is the leading cause of death (Bauer, Briss, Goodman, & Bowman, 2014; Go et al., 2013; Moy, 2017; Santulli, 2013). Endothelial dysfunction is a precursor to the development of atherosclerosis and cardiovascular disease (Matsuzawa et al., 2013; Shechter, Shechter, Koren-Morag, Feinberg, & Hiersch, 2014). Antioxidants reduce oxidative stress which may improve cardiovascular health (Khan et al., 2014). Increasing antioxidant consumption has been shown to alter endothelial-dependent dilation (EDD), which is a marker of endothelial function (Sincer et al., 2015). A single meal high in antioxidants increases serum levels of antioxidants (Jensen et al., 2008) and may acutely improve cardiovascular health.

Legumes, such as chickpeas, are known to be high in antioxidants (Kou et al., 2013; S. Wang et al., 2011), and the consumption of chickpeas have been shown to help prevent coronary disease (Khyade & Jagtap, 2016). Sprouting legumes further increases their antioxidant content (Mendoza-Sánchez et al., 2016). Sprouting involves an enzymatic process that causes the release of stored material and begins embryonic cell division within the seed (Villacrés et al., 2015). Sprouting chickpeas increase their antioxidant capacity (Luthria, Singh, & D'souza, 2014; Ramesh & Swami, 2016), and specifically increase levels of selenium and isoflavonoids (Guardado-Félix et al., 2017).

Incorporating sprouted chickpea flour into pasta is a viable method to increase legume and antioxidant intake (Flores-Silva et al., 2015; Flores-Silva et al., 2014), and may acutely improve endothelial function. Therefore, the purpose of the current study

was to determine if replacing 40% of the semolina flour in pasta with sprouted chickpea flour would improve post-digestion endothelial function compared to post-digestion of 100% semolina flour pasta. We hypothesized that brachial artery flow-mediated dilation (FMD), a test of endothelial function, would be increased after consuming pasta made with 40% sprouted chickpea and 60% semolina flour vs. pasta made with 100% semolina flour.

3.3 Methods

Study population

All study procedures conformed to the provisions in the Declaration of Helsinki. Thirty-four participants signed informed consent forms approved by the Institutional Review Board of Montclair State University. Of the 34 participants who enrolled, three dropped out following the screening and two participants participated in the first visit but did not complete the second. Therefore, twenty-nine healthy adults participated in this study: 6 men and 23 women.

Experimental Protocol

Participants reported to the Exercise Science Laboratory at Montclair State University for an initial screening visit. A baseline blood pressure, height, weight, and body composition was determined. Participants completed a medical questionnaire form and a consent form. Participants were excluded if they had food allergies or previous diagnoses of cardiovascular disease, hypertension, malignancy, diabetes mellitus, or renal

impairment. Participants who used tobacco products, and those taking any medications with known cardiovascular effects were asked to refrain from use 8 hours prior to experimental and control visits.

Chickpea Sprouting Protocol

Goya dried chickpeas were used in this study. The chickpea sprouts were prepared in the food science laboratory at Montclair State University using a protocol described previously (Khattak, Zeb, Bibi, et al., 2007). In brief, the chickpeas were left submerged in water for 18 hours. The water was then drained and the chickpeas placed in a porous colander and rinsed with tap water for 2-3 minutes three times per day for 6 days. At the conclusion of the last rinse of days 2-7, the chickpeas were placed in a 0.56°C refrigerator overnight. After the final rinse on day 7, all the water was drained from the chickpeas. Chickpeas were then placed into an Excalibur Food Dehydrator (8250 Ferguson Ave, Sacramento, CA, USA) at 49°C for 15 hours. The non-sprouted chickpeas were discarded and the sprouted chickpeas were placed into a Vitamix Blender (8615 Usher Road, Cleveland, Ohio, USA) and made into flour.

Semolina and Sprouted Chickpea Pasta Protocols

40% sprouted chickpea flour was weighed and measured along with 60% of Caputo Semola Di Grano Duro Rimacinata Semolina Flour. 400g of the mixture was then combined along with 160 mL of water to form a dough. The dough was then placed in plastic wrap and left to sit for 10 minutes at room temperature. This dough was then formed into balls and placed into a KitchenAid Gourmet Pasta Press Attachment of a

KitchenAid machine using the rigatoni attachment (553 Benson Road, Benton Harbor, MI 49022). The pasta was left in the refrigerator at 0.6°C and left for 24 hours. Before each participant visit, the pasta was placed in 3 cups of boiling water for 5 minutes. The pasta was then weighed to 255.15 grams in a plastic container and was combined with 21.27 grams of salted butter for flavoring. The semolina pasta was made by The Caputo Semola Di Grano Duro Rimacinata Semolina Flour. 400g of the flour was combined with 118 mL of water to form a dough. The dough was then placed in plastic wrap and left to sit for 10 minutes at room temperature. The remaining procedure was the same as the sprouted chickpea pasta.

Experimental and Control Visits

Prior to the consumption of pasta, the participants were asked to fast for 4 hours (hr), avoid caffeine for 12hr, and avoid alcohol consumption, over-the-counter drug use, and strenuous physical activity for 24hr. Participants consumed each pasta sample two hours before FMD testing. This length of time is enough to allow the pasta to be digested and the carbohydrate content to be converted to glucose and be absorbed into the blood stream (Goñi & Valentín-Gamazo, 2003; Woolnough, Bird, Monro, & Brennan, 2010). No more than seven days was allowed between the experimental and control visits, and the visits were scheduled for the same time of day.

The brachial artery FMD protocol was performed as done previously (Lennon-Edwards et al., 2014; Matthews et al., 2013). Participants laid on an exam table with their left arm perpendicular to their body at heart level. An occlusion cuff was placed on the

forearm approximately 3 cm below the antecubital crease. A 5-13 MHz linear phased array ultrasound transducer (GE Vivid i; Healthcare, Jiangsu, China) was used to simultaneously collect longitudinally images of the brachial artery with continuous Doppler blood velocity. Data collection began after the participant rested for ≈ 15 min. One continuous video was recorded that contained a one-minute baseline period, a five-minute period with the cuff inflated to ≈ 200 mmHg, and a two-minute period post cuff deflation.

Continuous ultrasound video was transmitted to a computer at 30 frames/s by way of an S-Video connection. LabChart 8 software was used to store the collected video. Frame by frame measurements of brachial artery diameter was determined using custom designed automated edge detection software in National Instruments LabVIEW 13.0. Peak diameter was determined after applying a 1-s-wide median filter to each data point. FMD was expressed as a percentage change in comparison to the average baseline diameter. The Doppler blood velocity was also assessed frame by frame and used with diameter data to calculate shear rate area under the curve from cuff deflation to peak diameter. Shear rate area under the curve has shown to be an accurate indicator of blood vessel dilation (Veglia et al., 2014).

Statistical Analysis

Statistical power calculations determined that 20 participants would be needed to detect a 10% difference in FMD between conditions with 90% power and an alpha level

of 0.05. FMD between the experimental and control visits were compared using a paired t-test. Data is expressed as mean \pm SEM.

3.4 Results

A total of twenty-nine (6 males, 23 females) participated in this study. The participants were generally young (age 25.41 ± 1.51 yrs), of average body composition (height 164.66 ± 1.42 cm, weight 66.43 ± 2.29 kg, body fat $25\pm 2\%$, BMI 24.5 ± 0.17), and normotensive (SBP 116.1 ± 0.18 mmHg, DBP 77.1 ± 0.12 mmHg, MAP 90.1 ± 0.12 mmHg, HR 75.9 ± 0.16 bpm). There was no significance between the blood pressure or pulse values between visits 1 and 2: systolic ($p = 0.24$) Diastolic ($p = 0.28$) and pulse ($p = 0.22$). Baseline artery diameter was $3.03\text{mm} \pm 0.13$ for SCF and $3.05\text{mm} \pm 0.15$ for Semolina ($p = 0.90$). AUC shear rate was 48591.81 ± 6423.07 for SCF and 53463.16 ± 5425.20 for Semolina ($p = 0.23$). FMD was greater following SCF $10.28\pm 1.19\%$ vs semolina $7.87\pm 0.81\%$ ($p < 0.05$).

3.5 Discussion

The primary finding of this study was that partially replacing semolina flour in pasta with SCF flour increases post-consumption FMD. This effect was observed in healthy normotensive participants with no known vascular disease. This suggests that this simple dietary substitution could have positive cardiovascular health benefits for the general public. Numerous studies show that increasing endothelial function has been proven to protect against atherosclerosis (Busse & Fleming, 1996; Higashi, Maruhashi,

Noma, & Kihara, 2014; S. Kumar, Kim, Simmons, & Jo, 2014; Ntaios, Gatselis, Makaritsis, & Dalekos, 2013; Weiner et al., 2014). Whereas, endothelial dysfunction has been shown to precede cardiovascular disease (Busse & Fleming, 1996; Greyling et al., 2016; Ras, Streppel, Draijer, & Zock, 2013).

Germination significantly increases micronutrient content of legumes (Ramesh & Swami, 2016). Upon sprouting, chickpeas become a rich source of isoflavonoids (HaiRong et al., 2013). Gharachorloo et al., (2013) results show that in addition to isoflavonoids, sprouting caused the phenolic content in chickpea to increase, which causes the legumes antioxidant capacity to increase. Thus, grinding and making flour from chickpeas proves to be an excellent source of natural antioxidants (Gharachorloo et al., 2013). The improved FMD response following SCF consumption possibly suggests that the antioxidants from the sprouted chickpeas may be acutely affecting endothelial function.

By replacing some of the semolina flour with SCF, the pasta also has decreased carbohydrate and increased protein content. Decreasing the carbohydrate content has shown to negatively affect endothelial function (Jovanovski, Zurbau, & Vuksan, 2015; Merino et al., 2013; Schwingshackl & Hoffmann, 2013; Wycherley et al., 2016). SCF also increases the protein content of the pasta. An increase in protein content has also been shown to decrease endothelial function (Ferrara et al., 2006; Merino et al., 2013). Thus suggesting the altered macronutrient content of the SCF pasta is not the driving factor for the improved FMD. This increases the likelihood that the positive effect of SCF on FMD is due to increased antioxidant intake.

Many studies show that antioxidants help neutralize free radicals in the body (Khan et al., 2014; Peñas et al., 2015; Perni, Calzuola, A Caprara, L Gianfranceschi, & Marsili, 2014; Stein et al., 1999; S. Wang et al., 2011). Antioxidants such as flavonoids decrease platelet aggregation and superoxide production and increase the release of platelet-derived Nitric Oxide (Engler et al., 2004; Freedman et al., 2001). The dilatation observed with FMD analysis is endothelium-dependent and mediated by nitric oxide (NO) (DOSHI et al., 2001). Studies have previously compared the positive impact of antioxidants on FMD results (Eskurza, Monahan, Robinson, & Seals, 2004; Grassi et al., 2009; Harris et al., 2009, 2011; Heiss et al., 2007; Lacroix et al., 2016; Sincer et al., 2015). Our results show that antioxidants affected the results of FMD.

It is important that antioxidant-rich meals be consumed in order to prevent cardiovascular disease (Afshin et al., 2014; Bouchenak & Lamri-Senhadji, 2013). Sprouted chickpea flour starch content slowly releases into the bloodstream which reduces the risk of hyperglycemia (Osorio-Díaz et al., 2008). The substitution of pasta with sprouted chickpea flour is a viable intervention to improving public health (Cevallos-Casals & Cisneros-Zevallos, 2010; Fares & Menga, 2014; Messina, 1999; Świeca et al., 2013). This is evidenced by the improvement in antioxidant content upon sprouting legumes (Gharachorloo et al., 2013; Kou et al., 2013; Masood et al., 2014; Mendoza-Sánchez et al., 2016; Peñas et al., 2015; Pulido et al., 2000; Ramesh & Swami, 2016; Świeca & Baraniak, 2014). In addition, the nutrient content and vitamins are improved in sprouted legumes in comparison to non-sprouted legumes (Devi et al., 2015;

Y.-C. Li et al., 2014; Masood et al., 2014; Mbithi et al., 2001; Nakitto et al., 2015; Ramesh & Swami, 2016; Sangronis & Machado, 2007).

Limitations

It is interesting to see this effect if FMD with sprouted chickpeas. Although, it is unclear if antioxidants are the reason for the difference. Further investigation is needed to determine the mechanism for the difference in FMD results. The blood glucose values during digestion differ between chickpea and semolina flour (Woolnough, Bird, Monro, & Brennan, 2010). Although Woolnough, Bird, Monro, & Brennan, (2010) shows that blood glucose values stabilize after 2 hours of digestion. Increasing the protein content may have altered the time required for digestion and how this may have impacted FMD. Our findings show correlation with the results from single dose meals performed in other studies (Dalbeni et al., 2015; Lacroix et al., 2016). Although, there was a lack of analysis of endothelial-independent dilation (Pryer, Vrijheid, Nichols, Kiggins, & Elliott, 1997). Increasing the starch content of chickpea flour could have possibly slowed down digestion and affected FMD results (Goñi & Valentín-Gamazo, 2003). Although, as evidenced by Goñi & Valentín-Gamazo, (2003) after 2 hours of digestion the blood glucose reading of chickpea flour was similar to that of the baseline.

Conclusion

The results of this study suggest that substituting 40% of the semolina flour in pasta with sprouted chickpea flour improves endothelial function in healthy adults. This improved vascular function is possibly due to an increase in antioxidants present in the

pasta. Thus, our results suggest that replacing traditional flour with sprouted chickpea flour may be a beneficial and simple dietary intervention for improving cardiovascular health.

3.6 Tables and Figures

Table 1 Subject Characteristics

Baseline Characteristic	Participants
N (M/W)	29 (6/23)
Age (yr)	25.4± 0.29
Height (cm)	164.7± 0.27
Body Mass (kg)	66.4± 0.43
BMI (kg/m ²)	24.5± 0.17
SBP (mmHg)	116.1± 0.18
DBP (mmHg)	77.1± 0.12
MAP (mmHg)	90.1± 0.12
Heart rate (bpm)	75.9 0.16

Screening subject characteristics for the 29 participants (mean ± SE). BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; MAP, mean arterial pressure.

Table 2 Nutrient Content per 100g of flour

	Chickpea flour	Semolina, unenriched
Water (g)	10.28	12.67
Energy (kcal)	387	360
Protein (g)	22.39	12.68
Total lipid (fat) (g)	6.69	1.05
Total carbohydrate, (fiber included) (g)	57.82	72.83
Fiber, total dietary (g)	10.8	3.9
Fatty acids, total saturated (g)	0.693	0.150
Fatty acids, total monounsaturated (g)	1.504	0.124
Fatty acids, total polyunsaturated (g)	2.983	0.430

Nutrient values according to United States Department of Agriculture

(USDA) Food Composition Database

Table 3 Vascular Measurement

	SCF	Semolina
Brachial Artery FMD (mm Δ)	0.30 ± 0.003	0.23 ± 0.002
Baseline brachial artery diameter (mm)	3.03 ± 0.13	3.05 ± 0.15
Peak brachial artery diameter (mm)	3.33 ± 0.13	3.28 ± 0.15
AUC shear rate	48591.81 ± 6423.07	53463.16 ± 5425.20

Vascular measurement responses to pasta samples according to flour composition (mean ± SE)¹. FMD, flow-mediated dilation; SCF, sprouted chickpea flour; AUC, area under the curve.

Figure 1 Flow-mediated dilation Semolina vs SCF

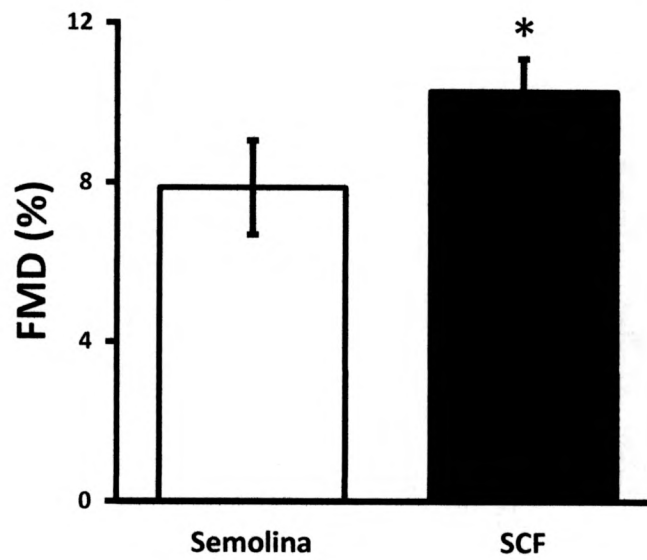


Figure 1. Flow-mediated dilation 2 hours post consumption of 100% semolina flour pasta (semolina) and 40% sprouted chickpea and 60% semolina flour pasta (SCF). FMD, flow-mediated dilation; SCF, sprouted chickpea flour. * $p < 0.05$. Mean \pm SEM.

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