Reducing food losses and improving the quality of locally produced spinach

by

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B.S., Aristotle University of Thessaloniki, Thessaloniki, Greece, 2011
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Abstract

Food loss and waste (FLW) constitute a substantial problem for global food security. Particularly, fresh produce constitutes 44% (by weight) of the global FLW. FLW of fresh produce occurs throughout the food chain, from production to the consumer. In the Central U.S., many small-acreage fruit and vegetable growers are utilizing high tunnels, which have been successful at increasing the yield of several crops. However, little is known about the effect of this production system on FLW. Moreover, small acreage producers have limited access to postharvest handling resources like refrigeration, which can lead to FLW during storage. Finally, consumer dissatisfaction is one of the main drivers of postharvest food waste in fresh produce. The overall objective of this dissertation was to investigate interventions for reducing FLW of locally produced spinach (*Spinacia oleracea* cv. Corvair). The effect of the high tunnel production system on preharvest losses, quality at harvest and during storage, consumer acceptability, and shelf life of spinach were examined and compared to the open field production system. We also evaluated the effect of passive Modified Atmosphere Packaging (MAP) on the quality and shelf life of locally grown spinach stored at non-optimum temperatures of 13 and 21 °C. Field experiments were carried out at the Kansas State University Olathe Horticulture Center from 2014 to 2017. We utilized a systems approach with six replications for each growing system. Shelf life experiments were performed at near-optimum and non-optimum temperatures. Spinach produced in high tunnels consistently demonstrated significantly higher marketable yield and higher percent marketability when compared to spinach produced in the open field. Both production systems produce spinach of premium physical and nutritional quality. There were no differences between the two treatments when spinach was stored at 3 °C. However, high tunnel spinach demonstrated improved postharvest behavior at 13 °C due to reduced respiration and yellowing rate and increased water content when compared to the open field. Consumer acceptability and sensory characteristics of spinach grown in the two systems were evaluated using a consumer study and descriptive sensory analysis. Consumers preferred spinach produced in high tunnels in terms of overall liking, flavor liking, and texture liking when compared to the open field and non-local spinach. Descriptive analysis showed that locally grown spinach had a higher intensity of attributes that indicate premium quality, such as green color and green/spinach flavors. BreatheWay® technology was investigated for the passive MAP experiments and spinach was stored at non-optimum temperatures. Spinach stored in MAP demonstrated a storage life extension, due to a slower rate of yellowing and water loss. The results of this work indicate that high tunnel production can reduce the FLW of spinach and produce a crop of high organoleptic quality that is preferred by consumers. Passive MAP has the potential to extend the storage life and maintain the quality of spinach stored at non-optimum temperatures. FLW is a complex challenge for the global food system and its reduction requires multidisciplinary collaboration, innovation and an approach tailored to the specificities of the various food chains.

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Acknowledgements

I would like to express my gratitude to my major advisor Dr. Pliakoni for her continuous support and guidance. Dr. Pliakoni prepared me for my future career and provided me with the maximum support and exposure to opportunities that a Ph.D. student can get. Thanks to her, I got involved in unique projects that were out of the strict scope of my program of study, which helped me to develop a multitude of skills and achieve enormous professional growth in the last 4 years. I will never forget what Dr. Pliakoni once told me: "My job is to make you succeed in your Ph.D. and your future career. My own success as a professor is bonded to your success".

Besides my advisor, I would like to thank my co-advisor Dr. Cary Rivard and my Ph.D. committee members (Dr. Jeff Brecht, Dr. C.B. Rajashekar, Dr. Shehbaz Singh) for their great support and invaluable advice. I am particularly thankful to Professor Jeff Brecht for all the help and for providing me with his postharvest wisdom during the last 4 years. I feel honored to have Dr. Brecht in my Ph.D. committee. I would like to acknowledge Dr. Shehbaz Singh for providing me with the expertise and the materials for my MAP experiments and for his sincere interest in my academic and professional success. Without Dr. Singh, I would not have been able to accomplish successfully a substantial part of my experiments.

I want also to acknowledge Dr. Martin Talavera and Dr. Marianne Swaney-Stueve for introducing me to the field of consumer and sensory studies. The knowledge I acquired from their classes and our research collaboration transformed my perception of fresh fruit and vegetable quality and was an inspiration for future collaborative projects between our fields.

I want to recognize here Kelly Gude and Josh Maher, both soon to be Drs., for being day-in-day-out on the campus. Having them go through the same experiences made the Ph.D.

struggle easier. I would like also to thank my lab mates and colleagues Tricia Jenkins, Joe Rundquist, and Dr. Helena Stanley, and the OHREC crew for all their help in my projects.

Finally, I would like to express my deepest gratitude to my family. I would like to particularly thank my wife Lindsey, for her love, understanding, and for making my life brighter.

Dedication

This dissertation is dedicated to my mother Paraskevi Zolota, who taught me patience and perseverance.

Chapter 1 - Introduction

"*Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences*" (FAO, 1996). According to this definition, the main elements of food security are: i) sufficient availability of food, ii) equitable access to food, iii) adequate food utilization through a nutritious diet and access to clean water and health services, iv) political, economic and social stability that ensures access at all times (FAO, 1996). Currently, approximately 1 billion people are not able to fulfill their basic caloric needs and around 2 billion people are experiencing micronutrient deficiencies (Barrett, 2010). Concurrently, the global per capita caloric intake has been steadily increasing, demonstrating a 20% increase from 1970 to 2011 (Alexandratos and Bruinsma, 2012; Sans and Combris, 2015) and in the same period, the world population has doubled (Kong, 2018). The global food demand is continuing to rise due to the increase in global population and the improvement of the standard of living around the world (Tilman et al., 2011; Mc Carthy et al., 2018; Porat et al., 2018). Specifically, the world population is expected to rise above 9 billion (UN DESA, 2017), while the global food demand is projected to increase between from 59% (Valin et al., 2014) to 110% (Mc Carthy et al., 2018) by 2050. This reality has fueled concerns that the current global food system is unsustainable and that it will eventually fail to provide food security for the future generations (Suweis et al., 2015; Myers et al., 2017; Vandermeer et al., 2018; Saint Ville et al., 2019). Globally, agricultural production systems have to deal with a combination of challenges. These include a decrease in the available natural resources, such as arable land (Bai et al., 2008; Foley et al., 2011), water (Rosegrant et al., 2009; Godfray et al., 2010; Foley et al., 2011), fossil fuels and their derivatives (Godfray et al., 2010; Abas et al., 2015; Amundson et al., 2015). Moreover, recent reports indicate that climate change has already had an adverse impact on agriculture leading to reductions in yield and quality (Campbell et al., 2016; Ochieng et al., 2016). While there have been major developments in controlled environment production, the majority of the agricultural production is greatly dependent on the natural climate conditions and factors such as solar radiation, temperature and rainfall (Rosenzweig et al., 2001). As a result, the increase in climate change-related phenomena such as temperature extremes, drought, flooding and other extreme weather effects (Barros et al., 2014) are reducing the stability of the global food systems (Wheeler and Von Braun, 2013). A manifestation of the increased instability of the global food system was the 2008 food crisis accompanied by food riots across the world (Schneider, 2008; Bellemare, 2015). Achieving food security poses a multidimensional challenge (Fouilleux et al., 2017) that requires progressive policy reforms and investment in fields such as education and research, rural infrastructure and resource management (Rosegrant and Cline, 2003). This dissertation will focus on research efforts examining methods and approaches for increasing the availability of high quality and nutritious fresh fruits and vegetables.

The majority of the efforts for increasing food availability have focused on increasing the productivity of agricultural systems (Pretty et al., 2010; Tilman et al., 2011; Grafton et al., 2015; Fouilleux et al., 2017). Estimations for the production increase needed to meet the expected future food demand vary from 60% (Alexandratos and Bruinsma, 2012) to 70-100% (Pretty et al., 2010). While ensuring agricultural productivity is crucial, reducing food losses and waste (FLW) should be an integral component of the strategy for achieving food security. FLW occurs throughout the food production and distribution chain and constitutes a substantial problem in regards to food availability (Tomlinson, 2013; Xue et al., 2017; Porat et al., 2018). Food losses and food waste, describe the quantitative and qualitative reduction of food produced for human consumption (Gustavsson et al., 2011; Lipinsky et al., 2013). Food losses involve the food being physically lost or inedible due to deterioration/spoilage while food waste involves discarding edible food due to unacceptable quality (e.g. irregular shape) (Lipinsky et al., 2013). FLW occurs during every step of the food system including production, postharvest handling, storage and transportation, processing and packaging, distribution, retail and consumption (Gustavsson et al., 2011). Each year, approximately 30% percent of the food produced for human consumption is lost or wasted (Gustavsson et al., 2011; Lipinsky et al., 2013; Myers et al., 2017). It is estimated that approximately 614 kcal per capita is lost or wasted every day globally in the postharvest chain (Kummu et al., 2012) while in the U.S., the amount is approximately 1,249 kcal per capita (Buzby et al., 2014). If the amount of FLW is reduced in half by 2050, the food system will need to produce 1314 trillion kcal less to meet the projected food demands (Lipinsky et al., 2013). Reducing FLW is a sustainable approach for increasing food availability (Kader, 2005; Lipinsky et al., 2013; Shafiee-Jood and Cai, 2016; Xue et al., 2017). Increasing agricultural productivity without reducing losses and waste is costly in regards to financial and natural resources as well as environmental degradation (Papargyropoulou et al., 2014; Shafiee-Jood and Cai, 2016). The cost of global FLW in 2007 was estimated at $750 billion (FAO, 2013) and this cost includes not only the food production, storage and transportation but also the waste disposal (Papargyropoulou et al., 2014). FLW landfill disposal leads to methane and carbon dioxide production (Papargyropoulou et al., 2014) with carbon dioxide emissions from FLW reaching 2.2 Gt in 2011 (Porter et al., 2016). Specifically, the greenhouse gas emissions from FLW is approximately a quarter of the total emissions from agricultural production (Searchinger et al., 2019).

Fresh fruits and vegetables constitute the largest portion of the food wasted or lost (Parfitt et al., 2010; Gustavsson et al., 2011; Shafiee-Jood and Cai, 2016; Porat et al., 2018). In fact, it is estimated that produce losses reach up to approximately 44% by weight of the total FLW (Lipinsky et al., 2013). The high amount of the FLW occurring in fresh fruits and vegetables is reflecting their biological nature. Fresh fruit and vegetables consist of living respiring plant tissue and are subject to biological deterioration, which constitutes them as highly perishable commodities (Kader, 2002). Reducing the losses and waste of fresh produce is of particular importance not only because of the severity of this issue, but also due to the value of these commodities in regards to human health (Kader, 2000; Rajashekar et al., 2009; Slavin and Lloyd, 2012; Liu, 2013). The losses and waste of fresh fruits and vegetables are not only quantitative but also involve invisible to the consumer characteristics such as nutritional content and flavor (Kader, 2000; Buzby and Hyman, 2012). FLW of fresh produce occurs in all parts of the chain in production, harvest, transportation, storage, processing, distribution, retail and consumption (Gustavsson et al., 2011; Gunders, 2012; Porat et al., 2018).

Losses during production, also referred to as preharvest losses, contribute considerably to the total amount of FLW. In Europe, North America and Oceania, preharvest losses reduce approximately 20% of the total productivity (Gustavsson et al., 2011). Among other causes, preharvest losses can be attributed to adverse weather conditions, pest infestation or plant diseases (Kantor et al., 1997; Buzby et al., 2011). They also include edible product left unharvested in the field due to labor shortages (McKissick and Kane, 2011; Gunders, 2012), low market prices (Kantor et al., 1997; Gunders, 2012), or for not meeting the commercial procurement standards (Kantor et al., 1997; Buzby and Hyman, 2012; Gunders, 2012; Lipinsky et al., 2013). Inappropriate harvesting methods or equipment malfunction can result in losses during harvesting (Kantor et al., 1997; Atanda et al., 2011; Kitinoja, 2013). The harvesting methods directly influence the postharvest losses of horticultural crops, with mechanical injury such as bruising, abrasion and cuts, which can increase the deterioration rate of the harvested commodity (Kader, 2000). Generally, the postharvest life, and therefore the losses, of fresh fruits and vegetables is highly determined by the crop quality at harvest (Weston and Barth, 1997). Harvest quality is determined by the conditions of the plant at, or just before harvest (Lee and Kader, 2000). The quality of non-climacteric commodities is at its maximum level on the day of harvest, and the crop quality is progressively decreasing due to natural deterioration after the harvest. Reported preharvest factors that can affect produce quality at harvest and the postharvest life of fresh produce include: genotype selection, environmental conditions, and the microclimate during growth and applied cultural practices (Weston and Barth, 1997; Mattheis and Fellman, 1999; Sams, 1999; Kader, 2000).

Postharvest losses can occur during storage, transportation, processing, distribution, retail and consumption (Kader, 2005) and may reach from 5% to 35% of the total fruit and vegetable production (Gustavsson et al., 2011). Postharvest losses of fresh produce are directly linked to the biological deterioration of these commodities and this deterioration is mediated by phenomena such as respiration, ethylene production and action, compositional changes, water loss, physiological disorders and pathological breakdown (Kader, 2005, 2013). The rate of deterioration is affected by the postharvest conditions and handling of the commodity (Lee and Kader, 2000; Prusky, 2011). Conditions such as non-optimum storage temperature and humidity, improper packaging, and physical damage during handling, will all increase the rate of deterioration and decrease the product quality leading to a shorter shelf –life (Kader, 2005; Hodges et al., 2011). Temperature abuse is the major factor contributing to food losses of fresh fruits and vegetables in postharvest chains, from the packing house to the consumer (Gustavsson et al., 2011; Prusky, 2011; Jedermann et al., 2014). Temperature dictates the rates of many of the aforementioned metabolic processes and physiological responses occurring in the plant tissues during storage (Sams, 1999; Kader and Saltveit, 2003), thus non-optimum storage or temperature abuse will shorten the shelf life and increase the losses of fresh produce (Bartz and Brecht, 2002; Kader, 2013; Vicente et al., 2014). An amount of postharvest losses of produce also occurs due to insect infestation (Kantor et al., 1997) or postharvest diseases (Wilson and Wisniewski, 1989).

Postharvest waste of fresh fruits and vegetables mainly occurs due to strict commercial cosmetic standards (Kantor et al., 1997; Gunders, 2012; Kyriacou and Rouphael, 2018) and it varies from 5% to 28% of the fruits and vegetables produced (Gustavsson et al., 2011). Harvested edible crops are frequently culled or even whole shipments are rejected due to strict quality standards set by major retailers (Buzby et al., 2011; Gunders, 2012). Product recalls due to food safety outbreaks/concerns, which lead to discarding large amounts of potentially-contaminated product, also contribute to waste of fresh produce since they involve discarding significant amounts of potentially-edible food (Kinsey et al., 2011; Pouliot and Sumner, 2013; Neff et al., 2015). While these recalls are of relatively low frequency, they can make a large contribution to FLW (Mena et al., 2011). Food safety outbreaks/recalls have an impact on the FLW of fresh produce further down the chain as well. In particular, they negatively affect consumer confidence in food safety leading to a reduction in consumption of the produce related to the outbreak product category e.g. leafy greens (Peake et al., 2014; Neff et al., 2015). Waste of fresh produce at the retail level also occurs due to overstocking, improper stock rotation (Buzby et al., 2011), whole package discarding when a single piece is spoiled (Lebersorger and Schneider, 2014; Buzby et al., 2015) and by discarding past “sell-by date” produce (Alexander and Smaje, 2008). In Europe, North America and Oceania, FLW of fresh produce at the consumer level range from 19 to 28% of the total produce purchased (Gustavsson et al., 2011). The main factor contributing to fresh produce losses at the consumer level is improper storage (Langen et al., 2015; Porat et al., 2018). Consumer food waste is a complex and multidimensional problem (Porat et al., 2018), but is mainly attributed to forgotten produce in storage (Langen et al., 2015; Waitt and Phillips, 2016), flavor dissatisfaction (Baldwin, 2002; Kader, 2005; Langen et al., 2015), visual quality dissatisfaction (Campbell et al., 2009), poor planning (Langen et al., 2015; Porat et al., 2018) and/or over purchasing (Langen et al., 2015).

There has been a steady increase in the demand and consumption of locally-produced food in the U.S. during the last two decades (Carey et al., 2009; Nie and Zepeda, 2011; Zumkehr and Campbell, 2015). There was a 140% increase in local food sales in the U.S. from 2008 to 2014, reaching $12 billion, and they are expected to reach 20 billion by 2019 (U.S. Department Of Agriculture., 2016). The term “local food” is used to describe food commodities that do not travel a long distance from the site of production to consumption (Watts et al., 2005; Chambers et al., 2007; Grebitus et al., 2013). Local food is typically produced by small-scale farmers (Francis, 2002; Horst and Gwin, 2018). This type of farmer, in the Central U.S., is cultivating on average 4.8 acres of land and a considerable amount is cultivating in less than 3 acres (Greater Kansas City Food Hub Working Group, 2015). Small acreage farmers in Kansas and the Central U.S. are frequently utilizing high tunnels for local fruit and vegetable production (Carey et al., 2009; Knewtson et al., 2010; Greater Kansas City Food Hub Working Group, 2015). A high tunnel is a greenhouse-like structure that is unheated and typically consists of metal hoops covered with a polyethylene film sheeting, with the crop production occurring directly in the soil. The main benefits of this production system are increasing yield (Waterer, 2003; Lamont, 2005), protection from the weather elements (Lang, 2009; Zhao and Carey, 2009; Hoppenstedt et al., 2019), and season extension (Borrelli et al., 2013; Galinato and Miles, 2015; Gude et al., 2018). Leafy greens are the most frequent crop group grown in high tunnels in the Central U.S. and the three most common crops are tomatoes, lettuce and spinach (Knewtson et al., 2010). Another common characteristic among small acreage farmers is that they have limited access to postharvest handling resources like optimum refrigeration conditions (Watkins and Nock, 2012; Greater Kansas City Food Hub Working Group, 2015) and many of the existing postharvest tools and techniques are not suitable for small-scale operations (Kitinoja and Kader, 2003). As a result, postharvest handling is a major challenge for this type of fruit and vegetable producer (Watkins and Nock, 2012; Greater Kansas City Food Hub Working Group, 2015; Watson, 2016). Developing postharvest tools and solutions that are suitable for application in small-scale farm operations may significantly reduce the FLW occurring in these production systems.

This dissertation will examine the utilization of the high tunnel production system by small acreage farmers as a tool for decreasing preharvest and postharvest losses as well as waste of locally-grown spinach. Additionally, it will examine the application of passive modified atmosphere packaging that is specifically designed for storage at non-optimum temperature as a solution for small acreage spinach farmers. The first chapter is a study on the effect of high tunnel production on preharvest food losses in fall-planted spinach. Preharvest losses were identified by investigating spinach yield and percent marketability, as well as the quality of spinach on the day of harvest, which was determined by a number of physical and nutritional quality parameters. The second chapter studies the effect of the high tunnel production system on the postharvest losses of spinach. Specifically, the effect of the high tunnel system was evaluated, in combination with optimum and non-optimum storage temperatures, on the shelf life, and the physical, organoleptic, and nutritional quality of spinach during storage. The third chapter consists of a study identifying the consumer acceptability and the sensory characteristics of spinach grown locally in Kansas, in open-field and high tunnel production systems as well as non-local purchased (commercially-grown) spinach that was grown in Salinas California, using a blind consumer test and descriptive sensory analysis. The fourth chapter presents a study on the effect of passive modified atmosphere packaging (MAP), using the BreatheWay® technology, on the postharvest losses of spinach when stored in non-optimum temperatures. The effect of passive MAP when spinach is stored at non-optimum temperatures, was evaluated in terms of the shelf life, physical, organoleptic, and nutritional quality of the product.

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Chapter 2 - Reducing preharvest food losses in spinach with the implementation of high tunnels

Abstract

Preharvest losses of fruits and vegetables constitute a considerable amount of the total food losses that occur in the food chain. Preharvest losses are typically related to a decrease and/or loss of marketable yield due to reduced crop performance and/or crop damage related to weather and/or pests and plant diseases. However, physical and nutritional quality are also important parameters that can contribute to food losses. There are numerous reports in the literature that indicate that small-acreage farmers in the U.S. can utilize high tunnels to increase the yield of various fruit and vegetable crops. However, it is unclear if the high tunnel production system affects the preharvest food losses of spinach, particularly in regard to physical and nutritional quality. The goal of this study was to examine the effect of the high tunnel production system on preharvest food losses of fall-planted spinach, Spinacia oleracea, cv. 'Corvair'. Comparative open field and high tunnel trials were conducted at the Kansas State University Olathe Horticulture Research and Extension Center from 2014 to 2017 (three growing seasons). A systems approach was utilized, consisting of six replications per production system and organic production practices were utilized. Spinach leaves were harvested at full maturity and the preharvest losses were evaluated in regards to yield, percent marketability and physical and nutritional quality. Spinach grown in the high tunnels had 126% - 528.6% higher marketable yield ($P < 0.001$) and 11.5% - 26.5% higher percent marketability ($P < 0.001$) when compared to open field production during the three years of the study. The high tunnels produced spinach with 30% to 50% larger leaves ($P < 0.001$) and 2.1% to 2.4% higher water content ($P < 0.001$) when compared to spinach grown in the open field. Spinach grown in the open field plots had significantly higher antioxidant capacity (ORAC & FRAP) in both years. There was an inconsistent effect on total phenolic content and ascorbic acid content, with these phytochemicals demonstrating significantly higher values for the open field plots in one of the two years examined. Our results indicate that using high tunnels for production of spinach can reduce the preharvest food losses as the results of increased productivity and marketability and premium crop quality.

Introduction

Food losses involve the reduction in mass and quality of edible food during the production, post-harvest, and processing stages of the food chain (Gustavsson et al., 2011). Food losses have been recognized as an important component in the challenge of feeding the world's growing population under the pressure of climate change (Challinor et al., 2017; Thornton et al., 2018) and continuously decreasing resource availability (Rosegrant et al.,2014). Approximately one-third of fruits and vegetables that are produced are never consumed (Kader, 2005). Preharvest losses can contribute towards a considerable amount of food losses and are often attributed to adverse weather conditions, pest infestation or plant diseases (Kantor et al., 1997). They may also include produce left in the field due to labor shortages or low market prices (Gunders, 2012), or for not meeting physical appearance criteria (Lipinsky et al., 2013). Preharvest food losses of fruits and vegetables can range from 7.1% up to 49.6% (Gustavsson et al., 2011). Food losses are mainly attributed to a loss of mass or decay. However, nutritional and organoleptic qualities are also important parameters that can lead to food losses (Kader, 2005). The quality of fruits and vegetables is determined by the conditions of the plant at, or just prior to harvest (Kader, 2000). This means that the quality at harvest is typically optimum and after that point, it cannot improve. Climatic conditions and cultural practices are amongst the preharvest factors that have a major effect on product quality (Weston and Barth, 1997; Kays, 1999; Sams, 1999; Kader, 2000).

A high tunnel is a type of unheated greenhouse that typically consists of metal hoops covered with a polyethylene film sheeting, with the crop production occurring directly in the soil. It is a production system that has the ability to alter the microclimate surrounding the crop (Lamont, 2005; Kadir et al., 2006; Zhao and Carey, 2009; O'Connell et al., 2012). High tunnels are utilized by growers in the United States and around the world primarily for their season extension and crop protection properties (Carey et al., 2009). Over the last decade, their use has swiftly increased in the United States(Jett, 2017), and they are particularly popular amongst vegetable growers in the Central U.S. (Greater Kansas City Food Hub Working Group, 2015). Benefits of this production system include early warm season crop production (Carey et al., 2009; Miles et al., 2012; O'Connell et al., 2012; Zhao et al., 2014; Vescera and Brown, 2019), control of specific insect species (Rogers et al., 2016; Kong et al., 2017), and disease incidence reduction (O'Connell et al., 2012; Hanson et al., 2016; Kong et al., 2017). In fact, high tunnels have been proposed as a financial risk management tool for horticultural crop growers similar to crop insurance (Belasco et al., 2013).

Most of the high tunnel research that has been reported is focused on small fruits and fruiting vegetable crops (Janke et al., 2017), However, spinach, *Spinacia oleracea* is a cool-season crop that is frequently grown in high tunnels across the United States (Carey et al., 2009). According to survey data, it is one of the most common crops grown in high tunnels in the Central U.S. (Knewtson et al., 2010). The optimum growing temperature for spinach is approximately 15.5 to 24°C (Ernst et al., 2012). In many parts of the U.S. including Kansas, spinach is often overwintered in high tunnels and remains productive throughout winter in

(Borrelli et al., 2013; Dos Santos Hecher et al., 2014; Buller et al., 2016; Drost et al., 2017; Orde et al., 2018), whereas the crop undergoes dormancy during the winter months in the open field. This makes spinach a suitable crop for production in high tunnel production systems as a complement to warm-season crops e.g. tomato (Donnell et al., 2011).

High tunnel production has shown to increase the total and/or marketable yield in a variety of crops (Lamont, 1999; Salamé-Donoso et al., 2010; Miles et al., 2012; Rogers et al., 2016; Kong et al., 2017; Vescera and Brown, 2019). Based on current definitions, an increase in the proportion of marketable products that are harvested in the high tunnel (percent marketability) would translate a reduction in preharvest food losses that are the result of insect infestation, disease, weather-related and/or physiological defects. The available literature indicates that high tunnels have demonstrated potential as a tool for reducing preharvest food losses by increasing the marketability of various crops (Waterer, 2003; O'Connell et al., 2012; Rogers and Wszelaki, 2012), but nothing has been reported that addresses this topic specifically. Researchers have reported improvements on overall/visual product quality by the implementation of high tunnels for a variety of crops, but frequently without defining which specific quality characteristics were improved (e.g. Blomgren, T. A., & Frisch, 2007; Carey et al., 2009; Lamont, 2005). Other studies have examined fruit physical traits like average fruit weight and size (Waterer, 2003; Kong et al., 2017), fruit weight and soluble solids concentration (Kadir et al., 2006; Medina et al., 2011), or they examine the quality in terms of defect occurrence/marketability (Waterer, 2003; O'Connell et al., 2012; Rogers and Wszelaki, 2012; Wallace et al., 2012). In the case of spinach, it has been reported that high tunnels can produce an average of 85.7% marketability during the growing season (Ernst et al., 2012). There is little information on the effect of high tunnel systems on the physical quality of the crop such as respiration rate or water content, despite numerous reports in the literature that these factors that are related to the shelf life of the commodity (Kader, 2005). In addition to physical quality, quality of fresh produce also includes flavor and nutritional characteristics (Kader, 2000). Palonen et al., (2017) reported that there was no effect on the phenolic content and/or antioxidant activity, and the Brix and acidity was decreased for raspberries grown in high tunnels. The results of Zhao et al., (2007a) indicated that spinach grown in high tunnels had significantly less antioxidant capacity as determined by the oxygen radical absorbance capacity (ORAC) assay. Similarly, it has been found that high tunnel production negatively affects the phenolic content of other leafy greens like lettuce (Xu et al., 2017) or Pac Choi (Zhao et al., 2009). In contrast, leafy brassica greens grown in tunnels demonstrated similar lutein content and significantly higher β-carotene content compared to the greens grown in open field (Reif et al., 2013).

The overall objective of this study was to examine the effect of high tunnel production on preharvest food losses in fall-planted spinach. Preharvest losses were identified by investigating spinach yield and percent marketability, as well as the quality of spinach at the day of harvest, which was determined by a number of physical and nutritional quality parameters.

Materials and Methods

Location

The experiment was conducted at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC), located in Olathe, KS (lat 38.884347 N, long 94.993426 W), USA. OHREC is in the hardiness zone 6A as defined by the U.S. Department of Agriculture (USDA) hardiness zone map with an average annual extreme minimum temperature of -12.2 to -15 °C (Cathey, 2013). Weather data were collected, for each year from October to April (Table 2.1), by a weather station on site (Mesonet, 2016). In Kansas, spinach is planted in the fall and harvested through the spring. The experiment was conducted over 3 years: 2014 - 2015 (year 1), 2015 – 2016 (year 2) and 2016 – 2017 (year 3).

Table 2.1 Monthly temperature range and average temperature, in Olathe, KS from October to April of 2014–15, 2015–16, and 2016-17 (Mesonet, 2016).

Year	**Month**	**Air temperature (°C)**		
		Max	**Min**	**Average**
2014-2015	October	20.7	8.2	14.4
	November	9.5	-2.5	3.5
	December	5.3	-1.7	1.8
	January	6.7	-6.7	0
	February	2.9	-8.8	-2.9
	March	15.6	1.2	8.5
	April	20.5	7.6	14
2015-2016	October	20.8	7.4	14.1
	November	15.0	3.6	9.3
	December	10.5	-1.1	4.7
	January	4.7	-6.3	-0.8
	February	10.6	-2.4	4.1
	March	17.4	3.4	10.4
	April	20.7	7.7	14.2
2016-2017	October	23.2	10.1	16.7
	November	17.2	4.2	10.7
	December	4.7	-6.6	-0.9
	January	6.2	-4.7	0.7
	February	14.0	-0.4	6.7
	March	15.1	3.5	9.3
	April	19.7	8.2	13.9

Experimental Design and Plant Material

Spinach (*Spinacia oleracea* cv. Corvair) was grown in two production systems; high tunnel and open field plots. High tunnel and open field plots were part of a long-term systems experiment that was established in 2002 and have identical cropping histories. The experimental design was arranged similarly to the systems design described by O'Connell et al. (2012) and was identical to Hoppenstedt et al. (2019). This systems design does not involve randomization of the plots because one of production systems compared is a permanent structure. Six identical high tunnels (1 rep per tunnel) and six identical open field plots (1 rep per plot) were utilized and had the same soil type, chase silt loam (pH= 6.3). The high tunnels were Quonset style with 1.5 m sidewalls and were 6.1 m wide and 9.1 m long (Stuppy, North Kansas City, MO., U.S.A.) and equivalent open field plots (6.1 m × 9.1 m) were adjacent to the high tunnels. The high tunnels had 8-mm twin-wall polycarbonate end walls and a single layer of 6-mil polyethylene roof and sidewalls. In coordination with organic certification, the high tunnel and open field replications were divided longitudinally and crop rotation was carried out throughout the study that included buckwheat and tomato during the warm-season, and spinach and oats during the cool season. In every growing season of the study, spinach plots were previously planted with buckwheat, which was preceded by oats the prior winter and tomato the prior summer. Each spinach plot consisted of an 8.5m long bed with 5 rows each. The distance between rows was 30 cm and the spinach was planted with 15 cm in-row spacing.

Cultural Practices

Typical cultural practices for organic spinach in the region were utilized (Buller et al., 2016). Spinach seeds were germinated and grown in 72 cell trays in a shade house until the development of two to four true leaves. The seedlings were fertilized with Organic Neptune's Harvest (Neptune's Harvest, Gloucester, MA, U.S.A.) at the recommended rate (30 ml per 3785 ml of water). No additional fertilizer was applied to the beds before or after transplanting the seedlings other than the residues of the buckwheat cover crop. Each year, the seedlings were transplanted in the experimental plots during the fall and were overwintered to spring. Planting occurred on 3 Oct 2014, 5 Oct 2015, and 10 Oct 2016. Irrigation was applied as needed, which was typically once per week during the fall, every 2 weeks during the winter, and 1-2 times per week during the spring. Organic insecticides were applied as needed including spinosad (Dow AgroSciences; Indianapolis, IN) and azadirachtin (Biosafe Systems, LLC; East Hartford, CT, USA).

The microclimate of the plots for both production systems was managed during nighttime according to the ambient air temperature while in the daytime sun exposure was also taken into consideration. During the nighttime, the high tunnel plots were covered with 33.9 g/m^2 row cover (Farmtek, Dyersville, IA, USA), when the forecasted temperature was below -6.5 °C, while the tunnel vents and sidewalls were kept closed when the temperature was below 4 °C. When the outside temperature reached, 4.5 °C the vents were kept constantly open and when the temperature was above 10 °C, the sidewalls were opened. For the open field system, the plots were covered with 33.9 g/m^2 row cover (Farmtek, Dyersville, IA, USA), when the ambient nighttime temperature was below 4.5 °C. During the daytime, the high tunnel plots were covered when the temperature was below -6.5 °C and when the temperature was below -1°C and cloudy or/and raining. The tunnel vents and sidewalls were kept both closed when the temperature was below 4.5 °C and when the temperature was below 10 °C and cloudy or/and raining. In sunny days and above 10 °C the tunnel sidewalls were kept open, while in partially sunny days the sidewalls were opened when above 21 °C and in cloudy/rainy days, they were opened when above 26.5 °C. For the open field system, the plots were kept covered during the day and night when the ambient temperature was below 1 °C. During sunny days when the temperatures were above 1 °C, the cover was removed, but the plots were kept covered at this temperature when it was partially sunny or cloudy/rainy. When the ambient temperature was above 4.5 °C, the open field plots remained constantly uncovered.

Data Collection

Yield

The harvest period was from November to April, and in year 2 harvesting was conducted until early May. During the winter months, there were harvest days when no spinach was available from the open field plots. Dates that have an asterisk (*) are ones in which only spinach grown in the high tunnel was harvested. Across the three years, the spinach was harvested 33 times in the high tunnel and 19 times in the open field. During year 1, spinach was harvested on 11/21, 12/5, *12/12, *1/16, *1/30, *2/13, *2/27, *3/11, 3/30, 4/6, 4/13 and 4/20. During year 2, spinach was harvested on 11/16, 12/4, 12/21, *2/29, 3/2, *3/21, 4/13, 4/25 and 5/4. During year 3, spinach was harvested on *11/16, 11/29, *12/16, *1/7, *1/25, *2/3, 2/23, *3/8, 3/22, 3/30, 4/12, and 4/21. Total and marketable yield were estimated from each plot by using data obtained from two sub-samples, which were each 0.28 m^2. Only the mature leaves were harvested including the petiole, while the immature leaves were left on the plant. Leaves with defects such as yellowing, holes or insect infestation were considered culls and their weight was recorded. The total yield and marketable yield were calculated per m^2 and percent marketability was calculated as (marketable yield/total yield) x 100%. During the first 8 harvests of 2014-2015, only marketable yield data were collected, thus the data for total yield, cull yield and percent marketability presented accounts only for the period 3/30 - 4/20.

Assessing Spinach Quality

Spinach quality was assessed on the day of harvest during years 2 and 3. Spinach leaves were sampled from 4 harvests during year 2 and five harvests during year 3. In year 2, the sampling dates were at 11/16, 12/4, 3/21 and 4/13, while in year 3, the sampling dates were at 11/29, 2/23, 3/22, 4/12 and 4/21.

During year 2, the quality analysis included six replicates per production system, which corresponded directly with the high tunnel and open field experimental design. In year 3, the first two harvests similarly included six replicates whereas the other three harvests consisted of three replications. Due to the limited supply of spinach, two of the replications from the field/tunnel study were aggregated for quality assessment. After being harvested, graded and weighed, spinach was transferred immediately to the postharvest physiology lab at the K-State Olathe campus in coolers and washed three times in ice-cold tap water. Next, the spinach leaves were centrifuged using a 5-gallon salad spinner (Chef Master 90005, China) for removing excess water before quality was assessed.

Respiration Rate

Respiration rate was determined by the closed system method as described by Jacxsens et al. (1999). Approximately 10 spinach leaves were placed in sealed glass jars (0.75 L Le Parfait, Villeurbanne, France) with a septum installed in the lid, for 60 minutes prior to the measurements. A portable gas analyzer (Bridge analyzer; Bedford Heights, OH, USA) was used to measure the amount of CO2 produced and the respiration rate was calculated as mg CO2/kg h.

Physical Quality

Physical quality was evaluated by measuring color leaf area, leaf density thickness measurements, leaf firmness, chlorophyll content, and water content. The measurements were performed in the same sequence using the same set of leaves for all the parameters.

Color measurements were determined using an A5 Chroma-Meter Minolta CR-400 (Minolta Co. Ltd., Osaka, Japan). For each experimental unit, the color of five leaves was measured on the upper side of the leaf, in two spots that were diametrically opposed to the leaf axis. Color results were expressed with the CIELAB color system, L* is lightness and h° is hue angle (McGuire, 1992).

The leaf area of five spinach leaves was measured using an LI-3100C Area Meter (LI-COR, Lincoln, NE, USA). The leaves were weighed and leaf density thickness was calculated by dividing the leaf weight by the leaf area. In year 2, leaf area and leaf density thickness were only measured in one harvest due to equipment-related issues.

The tenderness of the leaves was measured with a texture analyzer TA-58, TA.XT.plus (Texture Technologies Corp., Scarsdale, NY, USA), using a TA-91 Kramer Shear Cell (Texture Technologies Corp., Scarsdale, NY, USA) equipped with 5 blades. The midrib of 10 leaves was removed and the leaves were stacked and weighed. The force required to cut through the stack of leaves was measured as the peak force similarly to Prakash et al. (2000). The return distance of the 5-blade probe was 35mm, the test speed 1.7 mm/s and the return speed was 10 mm/s. Leaf tenderness was calculated as maximum force N per gram.

The extraction and quantification of chlorophyll content was performed according to the method described by Wellburn (1994). Samples of 0.3 g of leaf tissue were pulverized with 10 ml of methanol using a benchtop homogenizer (POLYTRON PT 1600 E, Kinematica AG, Luzern, Switzerland) and incubated in the dark at 4°C for 24 hours. After this period the supernatant was measured at 653 nm (chlorophyll b) and 666 nm (chlorophyll a) using a 96-well microplate reader spectrophotometer (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA). Total chlorophyll content was calculated using the following equations: Chlorophyll a (Chl a): [15.65 x (A666) – (7.34 x (A653)]; Chlorophyll b (Chl b): [27.05 x (A653) – (11.21 x (A666)] and Total chlorophyll content= Chl a + Chl b. The concentration was expressed as mg/100g FW.

Water content (WC) was measured by using 5 grams of fresh tissue that was sampled from five randomly selected leaves from each experimental unit. The fresh mass (FM) samples were weighed and consequently placed in a drying oven (Precision™, Thermo Fisher Scientific, Waltham, MA, USA) at 80 °C. After 24 hours, the dry mass (DM) was weighed. Water content was calculated by the equation provided by Agüero et al. (2008), WC(%)= [(FM-DM)/FM]x100.

Nutritional Quality

Nutritional quality was evaluated in terms of total phenolic content, antioxidant capacity and ascorbic acid content.

Spinach leaves were frozen with liquid nitrogen and stored at -20°C for analysis. The frozen samples were freeze-dried using a Harvest Right Freeze drier (North Salt Lake, UT, USA). All samples were pulverized using a pestle and mortar. The samples were extracted by mixing 20ml of acetone/water (1/1) solution with 0.2 grams of spinach powder. The mixture was shaken at 80 rpm for 1 hour using a 2314 Multi-Purpose Rotator (Thermo Fisher Scientific, Waltham, MA, USA) and consequently centrifuged (JA-17, Beckman Coulter, Palo Alto, CA, USA) at 11,300 rpm at 4°C for 20 minutes. The supernatant was analyzed for total phenolic content and antioxidant capacity. Total phenolic content was measured according to the procedure described by Singleton and Rossi (1965). Using a 96-well microplate reader (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA) at 750nm absorbance, the results are expressed as mg Gallic acid equivalent in 100 g fresh weight basis (mg GAE/100g FW). Antioxidant capacity was determined by measuring the Ferric Reducing Ability of Plasma (FRAP) and the Oxygen Radical Absorbance Capacity (ORAC). FRAP was measured according to the method described by Benzie and Strain (1996). Using the 96-well microplate reader with the spectrophotometer at 593nm, absorbance was determined against the Trolox positive control and expressed as micromolar

Trolox equivalent in 100g fresh weight basis (µmol TE/100g FW). ORAC was measured using 96-well microplate fluorometer, by the method described by Cao et al. (1993) and modified by Ou et al. (2001) and Prior et al. (2003). Antioxidant activity was expressed as micromolar Trolox equivalent in 100 g fresh weight basis (µmol TE/100g FW).

For measuring vitamin C, two g of homogenized spinach tissue from each experimental unit was added to 20 ml of acid solution (6% metaphosphoric acid/ 2N glacial acetic acid) according to the method of Klimczak and Gliszczynska-wiglo (2015). The samples were frozen at -20 ˚C and analyzed later using Ultra Performance Liquid Chromatography (UPLC). For the analysis, the samples were thawed and centrifuged at 7850 rpm for 10 min (JA-17, Beckman Coulter, Palo Alto, CA, USA). The supernatant was diluted with the acid solution and filtered using a 0.2µm PTFE syringe filter (VWR International, Radnor, PA, USA). The analysis was performed by injecting 5 µL of the diluted sample in an Acquity Waters UPLC (Waters Corp., Milford, MA, USA) equipped with an Acquity BEH C18 column (Waters Corp., Milford, MA, USA). The flow rate was set at 0.2 mL/min and the linear gradient of mobile phase was composed by 5 mM potassium phosphate monobasic (KH_2PO_4), pH 2.65 with 0.1% of formic acid (solution A) and methanol with 0.1% of formic acid (solution B). The linear gradient of mobile phase was used according to the program 5-15% A in 1 min, 15-35% for the next min and return to initial conditions in 4 min. Ascorbic acid was detected at 245 nm using the Acquity photodiode array detector (PDA) (Waters Corp., Milford, MA, USA). The quantification of the samples was performed by a five-point standard curve (2.5 µg/mL - 50 µg/mL) with purified ascorbic acid (assay percentage range ≥99.0 %, Fisher Scientific, Hampton, NH, USA) as a standard.

Statistical Analysis

All analyses were conducted using the statistical language R version 3.4 (R Foundation for Statistical Computing, Vienna, Austria). The equality of variance of the data was investigated using Levene's tests and unequal variances were found ($P < 0.05$). Therefore, the Welch one-way analysis of variance (ANOVA) was used instead of the traditional F-Test ANOVA in order to account for the heterogeneous variances (Glass et al., 1977). This type of analysis is not affected by unequal sample size and is also robust against the absence of normality in the residuals of the models as long as Pearson's moment coefficient of skewness is smaller than two (Lix et al., 2008). In the dataset of this study, large skewness ($\gamma 1 > 2$) was found only in the cull yield variable for the open field system in year 2 and 3 (8% of the yield data), which means that the dataset was not highly skewed. Data from the three years were combined and analyzed using Welch ANOVA to account for unequal variances. Based on the presence of significant interactions between the treatment being tested (production system) and year, the combined data was separated for presentation in the tables for all parameters. The yield and quality data were analyzed using Welch ANOVA with a Holm adjustment for the P-value for determining if significant differences ($P < 0.05$) existed.

Results

Yield

We observed that total yield ($P < 0.001$), marketable yield ($P < 0.001$), and percent marketability ($P < 0.01$) were affected by the year. In addition, the production system affected total yield, marketable yield, cull yield and percent marketability ($P < 0.001$; Table 2.2). A year*system interaction occurred for total yield, cull yield and percent marketability ($P<0.001$, Table 2.2), thus each year was analyzed separately. In year 1 (2014-2015), the high tunnels produced 177% higher marketable yield when compared to the open field plots ($P < 0.001$; **Error! Reference source not found.**). During the spring growing period, the total yield between the two production systems was similar (high tunnel 1065.8 vs open field 1103.6 g/m^2), but the cull yield for spinach grown in the open field plots was 169% higher than spinach grown in the high tunnels (high tunnel 176.3 vs open field 474.5 g/m^2 $P < 0.001$). As a result, spinach grown in the high tunnels demonstrated 26.5% higher percentage marketable yield compared to spinach grown in the open field plots (high tunnel 83.5 % vs open field 57.0% $P = 0.001$). During the year 2 (2015-2016), the high tunnels produced 84.6% higher total yield ($P < 0.001$), 126% higher marketable yield ($P < 0.001$) and 12.1% higher percent marketability ($P < 0.001$) when compared to the open field system (**Error! Reference source not found.**). In this year, there was no difference in cull yield between the two production systems (**Error! Reference source not found.**). During year 3 (2016-2017), high tunnel production demonstrated 443% higher total yield ($P < 0.001$) and 528.6% higher marketable yield ($P < 0.001$) when compared to open field production (**Error! Reference source not found.**). In year 3, high tunnel production had 11.5% higher percentage marketability when compared to open field production ($P < 0.001$; **Error! Reference source not found.**).

Table 2.2 Probability values reflecting the effects of the year (Y) and production system (S) on the total, marketable and cull yield, as well as percent marketability of spinach grown in high tunnel and open field trials from fall 2014 to spring 2017.

Interactions^z	**Total yield**	**Marketable yield**	**Cull yield**	**% Marketability**
Year (Y)	<.001	<.001	NS	<.01
Production system (S)	<.001	<.001	<.001	<.001
Y x S	<.001	NS^y	<.001	<.001

^{z}Welch ANOVA was used to test which factors and interactions between factors had a significant effect on the examined field data (α=0.05).
^{y}NS = Non-significant

Table 2.3 Total yield, marketable yield, cull yield and percentage marketability of spinach grown in high tunnel and open field trials in Olathe, KS, in 2014–15 (Year 1), 2015–16 (Year 2), and 2016-2017 (Year 3).

Year[z]	Production system	Total yield (g/m^2)	Marketable yield (g/m^2)	Cull yield (g/m^2)	Marketability (%)
2014-15[y]	High tunnel	N/A	2115.1 **a**[x]	N/A	N/A
	Open field	N/A	764.6 **b**	N/A	N/A
	P value	NS[w]	<.001	<.001	.001
2015-16	High tunnel	2228.4 **a**	1464.5 **a**	763.92 **a**	65.7 **a**
	Open field	1207.4 **b**	647.3 **b**	560.14 **a**	53.6 **b**
	P value	<.001	<.001	NS	<.001
2016-17	High tunnel	2070.3 **a**	1604.8 **a**	465.5 **a**	77.5 **a**
	Open field	381.2 **b**	255.3 **b**	125.9 **b**	67.0 **b**
	P value	<.001	<.001	<.001	<.001

[z] Data is separated by year due to significant interactions observed between production system (S) and year(Y).

[y] During the first 8 harvests of 2014-2015, only marketable yield data were collected, thus the data for total yield, cull yield and percent marketability are not presented in this table.

[x] Within column and year means followed by the same letter are not significantly different by Holm adjustment test at the $P < 0.05$ significance level.

[w] NS = Non-significant.

Physical Quality

Respiration rate ($P < 0.01$), color ($P < 0.001$), leaf area ($P < 0.001$) leaf firmness ($P < 0.001$) and water content ($P < 0.001$) were affected by the year (Table 2.4). The production system had an effect on leaf area, leaf firmness, water content ($P < 0.001$ for all parameters) and chlorophyll content ($P < 0.05$), but did not affect respiration rate, color and leaf density thickness (Table 2.4). A significant year*system interaction occurred for color lightness ($P < 0.01$) and leaf firmness ($P < 0.001$) (Table 2.4). In year 2 (2015-2016), spinach grown in the open field plots had an approximately 20% higher respiration rate at the day of harvest when compared to spinach grown in the high tunnels ($P < 0.05$; Table 2.4). Spinach leaves produced in the high tunnels had 30% larger leaf area ($P < 0.05$) and 2.4% higher water content ($P < 0.001$) when compared to spinach grown in the open field plots (Table 2.5). In year 2, there were no differences in color, leaf density thickness, leaf firmness, and chlorophyll content at the day of harvest between the two production systems (Table 2.5). In year 3 (2016-2017), there were no differences in respiration rate, color and leaf density thickness between the two production systems (Table 2.5). Similarly to year 2, the spinach grown in the high tunnels had 50% larger leaf area ($P < 0.001$) and 2.2% higher water content ($P < 0.001$) when compared to spinach grown in the open field plots (Table 2.5). Spinach grown in the open field plots demonstrated significantly firmer leaves ($P < 0.001$) and had 31% higher chlorophyll content ($P < 0.001$) (Table 2.5).

Table 2.4 Probability values reflecting the effects of the year (Y) and production system (S) on respiration rate, color, leaf area, leaf density thickness, leaf firmness, chlorophyll content and water content of spinach grown in high tunnel and open field trials from fall 2014 to spring 2017.

Interactions[z]	**Respiration rate**	**Color L**	**Color Hue**	**Leaf area**	**Leaf density thickness**	**Leaf firmness**	**Chlorophyll content**	**Water content**
Year (Y)	<.01	<.001	<.001	<.001	NS[y]	<.001	NS	<.001
Production system (S)	NS	NS	NS	<.001	NS	<.001	<.05	<.001
Y x S	NS	< .01	NS	NS	NS	<.001	NS	NS

[z] Welch ANOVA was used to test which factors and interactions between factors had a significant effect on the examined physical quality parameters ($\alpha=0.05$).
[y] NS = Non-significant.

Table 2.5 Respiration rate, color, leaf area, leaf density thickness, texture, and water content of spinach grown in high tunnel and open field trials in Olathe, KS, in 2015–16 (Year 2) and 2016-2017 (Year 3). Each value represents the mean of measurements obtained from four harvests in Year 2 and five harvests in Year 3.

Year[z]	**Production system**	**Respiration rate ($mgCO_2/Kg\text{-}h$)**	**Color L**	**Color Hue**	**Leaf area (cm^2)**	**Leaf density thickness (gr/cm^2)**	**Leaf firmness (N/g)**	**Chlorophyll content (mg/100g FW)**	**Water content (%)**
2015-2016	High tunnel	89.3 **b**[y]	35.3 **a**	127.7 **a**	86.31 **a**	0.012 **a**	1.73 **a**	100.2 **a**	88.2 **a**
	Open field	110.0 **a**	35.2 **a**	127.1 **a**	65.02 **b**	0.011 **a**	1.74 **a**	96.6 **a**	86.8 **b**
	P value	<.05	NS[x]	NS	<.05	NS	NS	NS	<.001
2016-2017	High tunnel	81.4 **a**	36.0 **a**	126.5 **a**	51.63 **a**	0.012 **a**	1.25 **a**	230.2 **b**	90.1 **a**

	Open field	76.9 **a**	36.7 **a**	126.2 **a**	34.83 **b**	0.012 **a**	1.78 **b**	301.8 **a**	87.9 **b**
	P value	NS	NS	NS	<.001	NS	<.001	<0.001	<.001

[z] Data is separated by year due to significant interactions observed between production system (S) and Year (Y).

[y] Within column and year means followed by the same letter are not significantly different by Holm adjustment test at the $P < 0.05$ significance level.

[x] NS = Non-significant

Nutritional Quality

Total phenolic content ($P < 0.001$), FRAP ($P < 0.001$) and ascorbic acid content ($P < 0.05$) were affected by year (Table 6). The production system had an effect on total phenolic content, FRAP, ORAC, and ascorbic acid content ($P < 0.001$ for all the parameters) (Table 6). In year 2 (2015/2016) spinach grown in the open field plots had 60% higher FRAP values ($P < 0.001$), 30% higher ORAC values ($P < 0.001$) and 13% higher ascorbic acid content ($P < 0.05$), compared to spinach produced in the high tunnels (Table 6). There was no difference in total phenolic content between the two production systems (Table 7). In year 3 (2016/2017), similarly to year 2, spinach grown in the open field demonstrated 30% higher FRAP values ($P < 0.001$) and 20% higher ORAC values ($P < 0.05$) when compared to spinach produced in high tunnels (Table 7). In contrast, in year 3, spinach produced in the open field plots total had 40% higher total phenolic content ($P < 0.001$) compared to the spinach produced in the high tunnels and there was no difference in the ascorbic acid content between the two production systems (Table 7).

Table 2.6 Probability values reflecting the effects of the year (Y) and production system (S) on total phenolic content, antioxidant capacity, and ascorbic acid content of spinach grown in high tunnel and open field trials from fall 2014 to spring 2017.

Interactions^{z}	**Total phenolic content**^{y}	**FRAP**	**ORAC**	**Ascorbic acid content**
Year (Y)	<.001	<.001	NS^{y}	< .05
Production system (S)	<.001	<.001	<0.001	<.001
Y x S	<.001	NS	< 0.05	NS

^{z}Welch ANOVA was used to test which factors and interactions between factors had a significant effect on the examined nutritional quality parameters (α=0.05).

[y] NS = Non-significant

Table 2.7 Total phenolic content, antioxidant capacity, chlorophyll, and ascorbic acid content of spinach grown in high tunnel and open field trials in Olathe, KS, in 2015–16 (Year 2) and 2016-2017 (Year 3). Each value represents the mean of measurements obtained from four harvests in Year 2 and five harvests in Year 3.

Year[z]	**Production system**	**Total phenolic content (mg GAE/100g FW)**	**FRAP (µmol TE/100g FW)**	**ORAC (µmol TE/100g FW)**	**Ascorbic acid content (µg AA/g FW)**
2015-2016	High tunnel	65.1 **a**[y]	145.3 **b**	3676.9 **b**	672.3 **b**
	Open field	70.5 **a**	225.1 **a**	4888.9 **a**	759.7 **a**
	P value	NS[x]	<.001	<.001	<.05
2016-2017	High tunnel	49.7 **b**	230.2 **b**	3707.9 **b**	786.7 **a**
	Open field	70.8 **a**	301.8 **a**	4442.1 **a**	838.3 **a**
	P value	<.001	<.001	<.05	NS

[z] Data is separated by year due to significant interactions observed between production system (S) and year (Y).
[y] Within column and year means followed by the same letter are not significantly different by Holm adjustment test at the $P < 0.05$ significance level.
[x] NS = Non-significant

Discussion

The goal of this study was to examine the effect of high tunnel production on preharvest food losses in spinach, defined in terms of total productivity, percent marketability, and product quality at harvest. Preharvest losses may be directly related to crop productivity and the loss of marketable products due to environmental or biotic factors (Kader, 2000). Previous studies have demonstrated the ability of high tunnels to increase the total and marketable yield, compared to open field production, mainly for small fruit and fruiting vegetable crops (Salamé-Donoso et al., 2010; O'Connell et al., 2012; Rogers et al., 2016; Kong et al., 2017; Vescera and Brown, 2019).

There have been several studies involving high tunnel spinach production (Borrelli et al., 2013; Dos Santos Hecher et al., 2014; Drost et al., 2017; Orde et al., 2018), but none of them have compared open field production with the high tunnel system. In our study, the high tunnel system resulted in significantly higher marketable yield in three consecutive years and higher total yield in two of the three years.

In this research project, the high tunnels produced spinach throughout the winter months and there were 74% more harvest events in the high tunnels as compared to the open field plots across the three years. In the Central US, fall-planted spinach can remain productive in the high tunnel during the winter months whereas the plants experience winter dormancy in the open field (Buller et al., 2016). This trend has been observed by others (Drost et al., 2017; Orde et al., 2018), and suggests that high tunnels may be able to reduce preharvest losses that occur due to seasonal weather fluctuations, which are typical in the Central US. The number of harvests that we observed in the high tunnels was comparable to what Orde et al. (2018) reported for similar planting dates. In the open field, the spinach did not grow consistently and fell into dormancy during the winter, which led to "breaks" in open field production. This phenomenon has been reported to occur when the temperature drops below 2.2 °C (Ernst et al., 2012). During the course of our three-year study, six of the 21 months that were utilized for spinach production experienced average air temperatures that were below 2.2 °C. Unfortunately, we were not able to collect microclimate data from within the high tunnels, but the fact that harvesting occurred during the winter growing period suggests that the growing environment did not force the plant into dormancy. During winter, the environmental conditions in the high tunnels are less variable compared to the open field and high tunnels can maintain non-freezing temperatures when outside temperature are below freezing (Wien, 2009; Orde et al., 2018). Soil temperature in high tunnels can be warmer compared to the open field (O'Connell et al., 2012; Borrelli et al., 2013). Thus, high tunnels may reduce the negative impact of low temperatures during the winter months and maintain continuous growth of the spinach plants thereby reducing preharvest losses that occur due to seasonal weather fluctuations in temperate climates.

High tunnels create a protective microclimate that is beneficial throughout the year. They can mitigate extreme weather effects such as heavy rains and storms, strong winds and hail (Giacomelli, 2009; Lamont , 2005). Excessive rainfall is a considerable limiting factor for open field production in the Central U.S. (Hoppenstedt et al., 2019). Moreover, vegetables are highly sensitive to wind exposure, which can negatively impact yield and quality (Baldwin, 1988). In a previous study that was conducted at the Olathe Horticulture Research and Extension Center, the authors found that high tunnels consistently reduced wind speed, even when the sidewalls and vents were open (Zhao and Carey, 2009). This is of particular importance for the region since Kansas is amongst the top three states with high winds in the U.S.A (Lu et al., 2009). Limiting wind exposure also reduces exposure to dust, which is detrimental to the quality of leafy greens (Wallace et al., 2012) and of concern in regards to food safety (Miraglia et al., 2009). All of these factors may have contributed to higher total, marketable yield and percent marketability that was consistently observed for the high tunnel production system in these trials.

The percent marketability of any crop is a direct measure of preharvest losses. In our three-year study, the high tunnels produced consistently a higher percentage of marketable spinach compared to the open field production system. Other authors have reported similar findings for other crops. For instance, Rogers et al. (2016) reported 86% marketability for raspberry production in the high tunnel versus 71% at the open field. Wildung and Johnson (2012) reported 57-89% marketability for tomato production in the high tunnel versus 1-55% at the open field. Although these previous reports did not describe these parameters as preharvest losses per se, these studies provide evidence that food losses can be reduced by implementing high tunnel production systems. In our study, we did not categorize and record what led to the non-marketability of spinach leaves (e.g. yellowing, insect damage, leaf spots, etc.). However, future research on the effect of the high tunnel production system on these particular disorders would help to inform the body of knowledge, as seen in the high tunnel versus open field tomato production study by O'Connell et al., (2012).

Orde et al. (2018) reported that the yield of spinach grown in the high tunnel is affected by the growing season. We found a "year" effect in the yield parameters between growing seasons for both production systems but it was more pronounced for the open field treatment. This trend was most pronounced in year 3 (2016 - 2017) and the open field treatment performed considerably worse than the previous two years. Specifically, the range of the total yield across the two years, in which it was recorded during the whole season, in the high tunnels was 7% of the highest value whereas in the open field it represented 68% of the highest value observed. Similarly, in tomato, the high tunnel production system was reported to provide more consistent annual yields when compared to the open field (O'Connell et al., 2012). This supports the claim that the high tunnels provide a reliable production system for intensive specialty crop production especially when considering the unpredictable weather patterns that are common in the Central U.S. The results of our trials further support this conclusion by demonstrating for three consecutive years, high marketable spinach yields were recorded from the high tunnel plots. In our trials, the range of marketable yield in the high tunnels was 31% of the highest value whereas, in the open field, it represented 67% of the highest value observed. Our results for marketable spinach yield are in the range of what Ernst et al. (2012) reported for high tunnel production, approximately 1900 grams/m^2 during one growing season. The difference between a crop's theoretical maximum yield in a stress-controlled and resource abundant environment and the actual yield has been defined as the yield gap (Godfray et al., 2010; Van Ittersum et al., 2013). Closing this gap has the potential to substantially increase food supply (Godfray et al., 2010). We found that the percent marketability of spinach was higher in the high tunnel system during each year of this experiment. As a result, a smaller proportion of the total yield is lost due to culling, which results in reduced preharvest food losses for this crop. Increased percentage marketability for a crop can also result to a higher revenue and increased profit for growers (Sydorovych et al., 2013). The beneficial effect that the high tunnel system has on preharvest losses was particularly evident during the spring of 2015. In that period the total yield was similar for the two production systems, but the high tunnels demonstrated significantly lower cull yield, which resulted in a larger proportion of marketable crop.

The quality of fresh produce at harvest is related to the conditions during growth (Weston and Barth, 1997; Kays, 1999; Sams, 1999; Kader, 2000) and in our study, the production system had an effect on the physical quality of spinach. High tunnels produced consistently larger spinach leaves with higher water content than the open field. Increased evapotranspiration (ET) may induce water stress in plants (Moretti et al., 2010) and wind exposure and wind speed are major factors affecting ET (Foroud et al., 2010; Falamarzi et al., 2014). The continuous wind exposure of spinach grown in the open field may account for the lower water content when compared with the spinach grown in the high tunnels. In addition, wind exposure has been strongly correlated to a reduction in leaf area development (Grace, 1988). High tunnels can accumulate growing degree-days (GDD) faster than the open field during winter (Borrelli et al., 2013) and spring (O'Connell et al., 2012) and GDD can increase the rate of the leaf area development (Gabrielle et al., 1998). Increased leaf area is also linked to increased water content (Cutler et al., 1977). It has been also reported that the increased temperatures offered by the protected environment of a low tunnel resulted in increased leaf area specifically for growing spinach under cover (Gimenez et al., 2002).

The polyethylene film used to cover high tunnels typically blocks ultraviolet (UV) light (Costa et al., 2003). UV light exposure has been related to a smaller leaf area and increased leaf thickness (Huché-Thélier et al., 2016). The leaves from the spinach grown in the open field were smaller but there were no differences in leaf density thickness. However, the open field produced firmer leaves in year 3, which is an indication of thicker leaves (Fontana et al., 2018). The decreased water content in the open field spinach is also indicating thicker cell walls Increased leaf area is linked to increased water content (Cutler et al., 1977), which also indicates less tender leaves. Spinach grown in the open field demonstrated a higher respiration rate in year 2. Plants acclimate their respiration rate, according to their surrounding environment (Amthor, 1984; Plaxton and Podestá, 2006; Ryan, 1991) and as a response to abiotic stress such as UV irradiation and low-temperature exposure (Plaxton and Podestá, 2006). Spinach grown in the open field was more exposed to the elements, which can be reflected with the higher respiration rate immediately after harvest. Spinach with higher water content and lower respiration rate at harvest may demonstrate a longer shelf life (Gil, 2016), thus our results indicate that spinach produced in high tunnels could demonstrate a longer shelf life than the spinach grown in the open field. Clearly, there is a need to conduct further research in this area. There were no differences in the leaf color measured as L* and Hue between the two systems in both of the examined years, whereas higher chlorophyll content was observed for the spinach leaves grown in the open field in year 3. A similar disparity between surface color measurements and chlorophyll content was found in snap beans by Proulx et al. (2010) and was attributed to the fact that the colorimeter is measuring only small areas of the crop. Leaf chlorophyll content may change according to the light spectrum exposure (Huché-Thélier et al., 2016). The increase in chlorophyll in the spinach grown in the open field may be a result of differences in light exposure. Basil grown in the open field demonstrated higher chlorophyll content compared to basil grown in the greenhouse, which was attributed to increased light intensity (Kopsell et al., 2005). Chlorophyll content in spinach is also related to the temperature during growth (Yamori et al., 2005). In spinach, an increase of 5°C in growing temperature resulted in a significant decrease in chlorophyll content (Lefsrud et al., 2005). High tunnels maintain a higher temperature than the open field (O'Connell et al., 2012) and this may explain the results from our study. The chlorophyll values measured in year 2 were substantially lower for both treatments than the values measured in year 3. This phenomenon is similar to what Ors and Suarez, (2016) reported and was attributed to seasonality.

Preharvest losses may not always be quantitative and visible to the consumer and could include the production of crops of inferior nutritional quality. In our study, we found that the production system affected the nutritional quality of spinach. Spinach grown in the open field plots demonstrated consistently significantly higher antioxidant capacity, measured as ORAC and FRAP when compared to spinach grown in the high tunnels. However, this specific finding does not necessarily constitute the spinach grown in the high tunnels is of inferior quality. The ORAC values measured for the spinach grown in the high tunnel are more than double than the mean value that the USDA reported for fresh spinach, which was 1513 μmol TE/100g FW (Haytowitz and Bhagwat, 2010). Similarly, the FRAP values measured for spinach grown in the high tunnels are in the same range with the FRAP values reported for spinach by Machado et al. (2018). Zhao et al. (2007) reported considerably higher ORAC values for the spinach grown in the open field compared to spinach grown in the high tunnel. However, the values they reported were approximately 2 and 4 times the ones reported in this experiment for high tunnels and open field plots, respectively. This discrepancy can be attributed to different environmental conditions during the late summer growing season as well as the cultivar that was used (cv. Tyee). ORAC and FRAP content of vegetable commodities can demonstrate high variability between different cultivars, growing locations, and harvest season (Ou et al., 2002). Specifically, commercial spinach cultivars may demonstrate a 2-2.7 fold difference in ORAC values (Howard et al., 2002; Pandjaitan et al., 2005). Generally, a variation in nutritional quality is expected due to the interaction of environmental conditions and cultural practices (Shewfelt, 1990). Spinach grown in the open field demonstrated significantly higher ascorbic acid content in year 2, and while in the year 3 the content was not significantly different from spinach grown in the high tunnels. In leafy greens, the ascorbic acid content is negatively affected by cultivation in protected environments and is mainly attributed to increased temperature and reduced light intensity during plant growth (Lee and Kader, 2000; Lester, 2006). Variability in the ascorbic acid content of spinach should be expected, according to the environmental conditions between different growing seasons (Conte et al., 2008; Lester et al., 2013), which might explain our results. The ascorbic acid content measured in spinach from both production systems in this trial is at the same range reported for spinach by Gil et al., (1999) and Bergquist et al., (2006). Finally, in year 2 there were no significant differences in the total phenolic content between the two production systems, while in year 3 the spinach grown in the open field demonstrated significantly higher total phenolic content. Leafy green production in the open field typically results in higher accumulation of phenolic compounds compared to protected environment cultivation, mainly due to temperature and light intensity differences (Gil, 2016). Zhao et al.(2007b) reported a similar trend between open field and high tunnel production of lettuce. While the lettuce grown in the open field had higher total phenolic content compared to lettuce grown in the high tunnel, the differences varied across growing seasons. The phenolic content measured in this experiment was higher, for both treatments than the values reported by Asfi et al. (2012) and Chun et al. (2005), but lower than the values Bunea et al. (2008) reported for fresh spinach.

Conclusions

The results of this study show that production of spinach in high tunnels in the Central U.S. can reduce the preharvest food losses of this crop, particularly as they relate to productivity, marketability, and physical quality. This could establish high tunnel production as a method that can contribute to the wider effort for achieving sustainable intensification of agricultural systems (Garnett et al., 2013). High tunnels have the potential for improving food security and should be part of resilient food systems. Our results show that the implementation of high tunnel production systems are increasing food availability through increased yield and continuous production through the season in addition to reducing food losses. Specifically, the high tunnel system resulted in significantly higher marketable yield in three consecutive years and higher total yield in two of the three years. Availability is one of the principal components of food security and it involves the ability of agricultural systems to produce sufficient amounts of food (Schmidhuber and Tubiello, 2007). To our knowledge, this is the first study to report the effects of the high tunnel on the physical and nutritional quality of spinach. Both production systems produced spinach of superior quality as indicated by the physical and nutritional quality measurements at the day of harvest. Spinach grown in the high tunnels demonstrated characteristics, such as increased water content and reduced respiration, which indicates a potential for shelf life extension during storage. In addition, a recent report indicates that consumers preferred the spinach produced in high tunnels more than spinach grown in the open field in terms of overall liking, flavor, and texture (Batziakas et al., 2019b). Consumer flavor-related dissatisfaction is a growing issue in the fresh produce sector (Baldwin, 2002) and can contribute to postharvest food waste (Kader, 2005). Further research is needed for investigating the effect of the high tunnel production system on the postharvest food losses and waste of spinach. The development of food systems that focus on achieving food security should consider incorporating high tunnel production systems due to their pivotal role in reducing preharvest losses.

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Chapter 3 - Reducing postharvest food losses in spinach with the implementation of high tunnel production system

Abstract

Postharvest losses of fruits and vegetables during storage constitute a considerable amount of the total food losses that occur in the food chain. The extent of these losses is directly related to the postharvest conditions and handling of the commodity, with non-optimum transportation and storage temperatures being the most detrimental factor affecting quality. However, postharvest losses are also directly linked to the quality of the product on the day of harvest, and this quality is defined by the preharvest conditions during growth in the field. The goal of this study was to examine the effect of a high tunnel production system on the postharvest losses of spinach (Spinacia oleracea, ‘Corvair') when stored at optimum and non-optimum temperatures. Comparative open field and high tunnel trials were conducted at the Kansas State University Olathe Horticulture Research and Extension Center from 2015 to 2017 (two growing seasons). A systems approach was utilized, consisting of six replications per production system and organic production practices were utilized. Spinach leaves were harvested at full maturity and stored at 3 °C or 13 °C for 19 and 9 days, respectively. The postharvest losses were evaluated with regard to the shelf life, and the physical, organoleptic, and nutritional quality of the spinach during storage. During storage at 3 °C, in both years, spinach grown in the high tunnels had 1.4% to 2.4% significantly higher water content than spinach grown in open field plots. However, at this storage temperature, there were no other major differences in physical and organoleptic quality between the spinach grown in the two production systems. There was an inconsistent effect on total phenolic content, antioxidant capacity, and ascorbic acid content during storage at 3 °C. These nutritional quality parameters demonstrated significantly higher values for the open field plots during storage, but not in both seasons. During storage at 13 °C, in both years, spinach grown in the high tunnels had 1.2% to 2.3% higher water content than spinach grown in the open field. In the second year, high tunnel spinach stored at 13 °C had lower respiration rate ($P < 0.05$) and slower rate of yellowing as indicated by higher chlorophyll content ($P < 0.001$), significantly lower lightness values, and significantly higher hue values than open field spinach. As a result, the spinach grown in the high tunnels demonstrated longer shelf life in year 1 and higher quality towards the end of shelf life in year two when compared to open field spinach when stored at 13 °C. Similarly to the storage experiment at 3 °C, there was an inconsistent effect on total phenolic content, antioxidant capacity, and ascorbic acid content during storage at 13 °C. Our results indicate that using high tunnels for production of spinach can reduce the postharvest food losses of this crop, particularly when stored at a non-optimum temperature, as a result of increased water content and decreased senescence rate.

Introduction

Global food demand is constantly increasing, primarily due to the rise in the world population (Tilman et al., 2011; Davis et al., 2016). Concurrently, agricultural systems have to deal with a constant decrease in natural resources (Flora, 2010; Suweis et al., 2015) and the adverse effects of climate change on agricultural production (Campbell et al., 2016; Ochieng et al., 2016), which is raising concerns about our ability to meet future food demand (Suweis et al., 2015; Myers et al., 2017). The majority of the efforts for meeting global food security are focusing on increasing the productivity of agricultural systems (Tilman et al., 2011; Grafton et al., 2015; Fouilleux et al., 2017). However, food losses and waste (FLW) occur throughout the production and supply chain and constitute a substantial problem with regard to global food security (Xue et al., 2017; Porat et al., 2018).

Food losses and food waste both involve the reduction of the edible food mass intended for human consumption (Gustavsson et al., 2011). The main difference between food losses and food waste is that losses involve the food being physically lost or inedible while waste involves discarding edible food due to unacceptable quality. Each year, approximately 30% percent of the food produced for human consumption is lost (Myers et al., 2017). Fresh fruits and vegetables comprise the biggest portion, covering 44% by weight of the total food lost or wasted (Lipinsky et al., 2013). Reduction of postharvest FLW of fresh fruits and vegetables is a sustainable approach for increasing food availability and contributing towards global food security (Kader, 2005; Lipinsky et al., 2013; Xue et al., 2017). Postharvest FLW in fresh fruits and vegetables is occurring in all parts of the chain, harvest, storage, processing, distribution, retail and consumption (Porat et al., 2018), in terms of both quantity and quality (Kader, 2005; Prusky, 2011).

After detachment from the mother plant, fresh produce is naturally deteriorating and its quality is progressively decreasing. This biological deterioration is mediated by phenomena such as respiration, ethylene production and action, compositional changes, water loss, physiological disorders and pathological breakdown (Kader, 2005, 2013). The rate of natural biological deterioration that leads to quality loss is affected by a variety of internal and external factors as well as the postharvest conditions and handling of the commodity (Prusky, 2011). Non-optimum storage temperature and humidity, improper packaging, and physical damage during harvesting and handling, will all increase the rate of deterioration and decrease the organoleptic quality of fresh fruits and vegetables (Kader, 2005; Prusky, 2011). While these losses can be associated with poor handling, consumer rejection, or senescence, the crop's postharvest life is mainly determined by its initial quality at harvest (Weston and Barth, 1997), which is affected by a variety of preharvest factors (Kader, 2000). Practically this means that, for non-climacteric commodities, the quality of the crop is the maximum possible at the day of harvest and will subsequently decline. Preharvest factors affecting the postharvest quality include genotype selection, environmental conditions, and the microclimate during growth and applied cultural practices (Weston and Barth, 1997; Mattheis and Fellman, 1999; Sams, 1999; Kader, 2000).

Leafy green vegetables are a highly perishable commodity group (Cantwell and Kasmire, 2002) and as a result, they demonstrate a considerable amount of postharvest losses during storage (Monaghan and Beacham, 2016). Like all fresh fruits and vegetables, the postharvest quality of leafy greens is affected by genotype selection, environmental conditions, and agricultural practices during growth. Specifically for leafy vegetables, light intensity and quality of light during growth affect the accumulation of bioactive compounds like anthocyanins (Gil, 2016), carotenoids (Li and Kubota, 2009), and ascorbic acid content (Shewfelt, 1990). It also affects parameters like the respiration rate (Plaxton and Podestá, 2006), chlorophyll content, and leaf thickness (Huché-Thélier et al., 2016). Likewise, the temperature conditions during growth may affect the organoleptic characteristics of leafy greens. For example, lettuce produced in temperate conditions is typically tender with subtle flavor (Weston and Barth, 1997). Contrarily, lettuce grown in higher temperatures may be more bitter (Peirce, 1987; Simonne et al., 2002; Bunning et al., 2010) and have tougher leaves (Peirce, 1987). High temperatures during growth of leafy greens, may also give rise to unwanted morphological changes like bolting (Zhao and Carey, 2009). Moreover, exposure to adverse weather conditions like wind and hail can have a detrimental effect on the appearance of leafy greens (Kays, 1999).

Water loss and yellowing due to chlorophyll breakdown are major factors that influence negatively the postharvest quality of leafy greens (Cantwell and Kasmire, 2002). Specifically, water loss of 3% in spinach and 3-5% in lettuce renders these commodities unmarketable (Bartz and Brecht, 2002). Pre-harvest water and nutrient management have a substantial effect on the postharvest quality of leafy greens (Gil, 2016). Water content is particularly important for the quality of leafy green vegetables since it is related to cell turgor, which directly affects textural properties (Sams, 1999). Improper water management may diminish leafy green quality parameters other than texture. Romaine lettuce grown in a deficit or a high irrigation environment demonstrated increased respiration after harvest and the lettuce produced under high irrigation conditions also demonstrated increased susceptibility to enzymatic browning (Luna et al., 2013).

Nutrient management affects the phytochemical content (Gil, 2016) and ascorbic acid content (Lee and Kader, 2000) of leafy greens. Application of organic amendments increased the water content but decreased glucosinolate, flavonol, and anthocyanin content in arugula (Selma et al., 2010). Generally, excessive nitrogen fertilization of leafy greens, including spinach, is linked to poor quality and postharvest disorders and can lead to decreased ascorbic acid and accumulation of nitrates (Mozafar, 1996; Weston and Barth, 1997). Moreover, excessive nitrogen application is associated with a decrease in vitamin C content in various leafy green commodities (Weerakkody, 2003; Xu et al., 2003). Spinach grown using organic methods demonstrated higher levels of ascorbic acid and flavonoids and lower levels of nitrates compared to conventionally grown spinach (Koh et al., 2012).

Spinach (*Spinacia oleracea* L.) is a leafy green vegetable of high nutritional value, particularly rich in antioxidants, polyphenols (Pandjaitan et al., 2005), and carotenoids (Bunea et al., 2008). It is a cool-season crop produced across the United States (Carey et al., 2009; Koike et al., 2011). Increasing consumer health awareness has led to an increase in spinach consumption in the U.S. (Morelock and Correll, 2008). This increase in spinach demand has subsequently led to increased spinach production in the United States (Morelock and Correll, 2008). Specifically, U.S. spinach production increased from 43,300 acres in 2007 to 53,029 acres in 2017 (FAO, 2019). Fresh appearance, tenderness, uniform green color, and absence of defects are the main components that characterize high-quality spinach (Wang, 2003). Similar to other leafy green commodities, the quality of spinach is affected by genotype selection, pre-harvest environmental conditions, production systems, and cultural practices.

The temperature range for optimum growth of spinach is approximately 16 to 24 °C (Wilcox and Pfeiffer, 1990; Ernst et al., 2012). However, spinach lutein, ϐ-carotene and chlorophyll content was reported to linearly decrease as the temperature during growth increased from 10 to 25 °C while the plant fresh mass increased linearly up to 20 °C but decreased at 25 °C (Lefsrud et al., 2005). Long days combined with high temperature will lead to spinach bolting and flower initiation, which is detrimental for spinach quality (Ikeda et al., 1995; Goreta and Leskovar, 2006). Specifically, when 10% of the spinach is bolting, the crop is rendered unmarketable and terminated (Brandenberger et al., 2004). High temperatures during spinach growth are also associated with the development of bitter taste (Fukuoka et al., 2019) and decreased sugar concentration (Gent, 2019).

A high tunnel is an unheated greenhouse-like structure, typically consisting of a metal frame covered with a single or double-layer plastic sheet (Carey et al., 2009). This production system has been used in the United States and worldwide, for the production of horticultural crops (Lamont, 2009). In the United States, there has been an increase in the utilization of high tunnels in the past 15-20 years, which has been linked to the rise in the demand of locally produced fruits and vegetables (Jett, 2017). This production system provides growers with the ability to manipulate the microclimate during crop production (Ogden and van Iersel, 2009; O'Connell et al., 2012; Wallace et al., 2012; Rogers et al., 2016), which subsequently has an effect on the quality of the commodity produced in this production system (Kong et al., 2017; Palonen et al., 2017; Batziakas et al., 2019b). The benefits of high tunnel production systems are well documented and they include: increase in yield (Hunter et al., 2012; Burlakoti et al., 2013; Rogers et al., 2016; Kong et al., 2017), season extension (Rowley et al., 2011; Garrett Owen et al., 2016; Gude et al., 2018; Orde et al., 2018), early warm season production (Hunter et al., 2012; Guan et al., 2018), protection from extreme weather (Lamont, 2009; Zhao and Carey, 2009; Gao et al., 2017), and disease incidence reduction (O'Connell et al., 2012; Burlakoti et al., 2013).

High tunnels have also demonstrated an ability to reduce preharvest food losses in spinach (Batziakas et al., 2019a). Particularly, high tunnel spinach production consistently resulted in higher marketable spinach yield and higher total spinach yield (Batziakas et al., 2019a). This production system can increase the total and marketable yield of a variety of crops compared to traditional open-field production (Salamé-Donoso et al., 2010; Miles et al., 2012; Galinato and Miles, 2015; Rogers et al., 2016; Kong et al., 2017; Batziakas et al., 2019a; Vescera and Brown, 2019). This practically means that the crop productivity is increased, also that more marketable product is being produced because high tunnels are protecting the crop from weather-related defects and disease incidence. However, the effects of high tunnels on postharvest losses of horticultural crops, as defined by shelf life and deterioration of physical, organoleptic, and nutritional quality during storage, is unknown. Generally, crops grown in a protected environment have been reported to be more perishable compared to open field crops, due to the absence of external stress during their development (Hewett, 2006). High tunnels have an effect on various fresh produce quality parameters on the day of harvest and have been found to affect specifically the physical (Batziakas et al., 2019a), nutritional (Zhao et al., 2007a, 2007c; Batziakas et al., 2019a), and organoleptic quality (Palonen et al., 2017; Batziakas et al., 2019b). All these quality attributes directly affect the overall postharvest quality and shelf life of fresh produce (Kader, 2000, 2005).

In the United States, the high tunnel production system is particularly popular among small acreage farmers (Carey et al., 2009; Belasco et al., 2013), and spinach is one of crops that is regularly cultivated by these growers (Carey et al., 2009; Knewtson et al., 2010; Buller et al., 2016). High tunnels allow continuous spinach production during the winter season by maintaining the temperature within the growth-conducive range of this crop (Borrelli et al., 2013; Orde et al., 2018; Batziakas et al., 2019a). This production system can increase the total yield and reduce the amount of cull product for this crop (Batziakas et al., 2019a). Spinach produced in high tunnels has larger leaves and increased water content and in some growing seasons demonstrated increased chlorophyll content and decreased respiration rate (Batziakas et al., 2019a), while it has consistently demonstrated reduced antioxidant content compared to open field production (Zhao et al., 2007c; Batziakas et al., 2019a). Moreover, a consumer study showed that consumers preferred spinach grown in high tunnels in terms of overall liking, flavor liking, and texture liking when compared to open field spinach (Batziakas et al., 2019b). Descriptive sensory analysis found that spinach produced in high tunnels was significantly sweeter and saltier compared to the open field spinach (Batziakas et al., 2019b).

The differences in morphology and composition between the crops produced using open field and high tunnel production systems may affect their postharvest quality and rate of deterioration (Kader, 2005, 2013). Spinach is a highly perishable crop with short shelf life even when stored in optimum conditions (Kader, 2002). The high perishability of spinach is attributed to its large surface-to-weight ratio and high respiration rate (Koike et al., 2011). Temperature and relative humidity are the most important parameters for maintaining quality and extending the shelf life of fresh produce (Kader, 2005). Particularly for spinach, the optimum storage conditions are 0 °C and 95-98 % RH (Suslow and Cantwell, 1999). However, it is typical for small acreage farmers to have a limited cooling capability (Watkins and Nock, 2012; Greater Kansas City Food Hub Working Group, 2015) and they frequently have to store produce at non-optimum temperatures (Watkins and Nock, 2012). Temperature control is the most crucial postharvest handling method for maintaining the postharvest quality of fresh produce (Prusky, 2011; Kader, 2013). Temperature regulates the majority of the metabolic processes and physiological responses occurring in fruits and vegetables during storage (Sams, 1999; Kader and Saltveit, 2003) including respiration (Kader and Saltveit, 2003) and water loss (Kitinoja and Kader, 2003; Prusky, 2011). The main problems associated with spinach storage at non-optimum temperatures are increased rates of water loss and yellowing (Koike et al., 2011). Spinach stored at 10 °C was unmarketable after 9 days of storage, while spinach stored at 2 °C for the same period maintained good quality and higher ascorbic acid content (Bergquist et al., 2006). Spinach stored at 20 °C demonstrated rapid yellowing, water loss, and wilting accompanied by accelerated loss of vitamins like vitamin C and vitamin B1 (Watada et al., 1987).

The overall objective of this work was to study the effect of two production systems, high tunnel, and open field, on postharvest losses of spinach. More specifically, we evaluated the effect of those production systems in combination with optimum and non-optimum storage temperatures on the shelf life, and the physical, organoleptic, and nutritional quality of spinach during storage. We hypothesized that the more favorable growing conditions in a high tunnel would result in improved postharvest quality and longer shelf life compared with open field spinach.

Materials and Methods

Experimental Design and Plant Material

Spinach (*Spinacia oleracea* cv. Corvair) was grown at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC) in Olathe, KS, USA. Spinach production was carried out in the open field and in high tunnel systems during a 2-year period: 2015-2016 and 2016-2017. Both production systems had identical cropping histories since their establishment in 2002. The plots followed a "systems" design identical to Hoppenstedt et al. (2019) and Batziakas et al. (2019a) meaning that the replications of each grown system were grouped per system, and the two groups of replications where placed next to each other in the field. Each production system was replicated six times with each replication consisting of a plot (6.1 m × 9.1 m) in the open field or in a high tunnel. Each of the 6 identical high tunnels used was Quonset style and was exactly the same structure used in Hoppenstedt et al. (2019) and Batziakas et al. (2019a). The cultural practices followed in this experiment are described in Batziakas et al. (2019a).

Mature spinach leaves for these experiments were harvested twice (4 December and 16 April) in year 1 (2015- 2016) and twice (29 Nov. and 12 April) in year 2 (2016-2017). Each time, leaves with defects such as yellowing, holes or insect infestation were discarded. After harvesting, the spinach was placed in coolers and immediately transferred to the postharvest physiology lab at the Kansas State University, Olathe campus. After washing three times in ice-cold tap water, the spinach was centrifuged using a 5-gallon salad spinner for removing excess water (Chef Master 90005, China). Subsequently, the spinach was inspected a second time and defective plant material was discarded. In year 1, the replications per treatment for the shelf life study corresponded to the six field replications per growing system. However, in year 2, the first harvest similarly included six replicates whereas the second harvests consisted of three replications per growing system due to limited supply of spinach. For each growing system, approximately 1 kg of spinach per replication was packed in produce bags. The bags were stored in environmental chambers (ThermoFisher Scientific Inc., Asheville, NC, USA), at 3 °C with 95% RH or 13 °C with 95% RH. The quality of the spinach was analyzed throughout storage, on days 0, 3, 9, 13, 17, and 19 for 3 °C, and on days 0, 3, 5, 7, and 9 for 13 °C.

Spinach Quality Analyses

Respiration Rate

The respiration rate of the spinach samples was measured using the closed system method (Jacxsens et al., 1999). Approximately 10 spinach leaves per replication were weighed and sealed in air-tight glass jars (0.75 L Le Parfait, Villeurbanne, France) equipped with a septum, for 60 minutes. The amount of CO_2 produced that accumulated in the void space of the jar during this period was measured using a portable gas analyzer (Bridge Analyzer; Bedford Heights, OH, USA) and the respiration rate was expressed as the rate of CO_2 production in mg/kg h.

Physical Quality.

Physical quality during shelf life was evaluated by measuring overall visual quality (OVQ), leaf surface color, leaf tenderness, water content, and chlorophyll content.

For each treatment, twenty leaves per replication were evaluated and rated visually, taking into account freshness, appearance, and color uniformity, similarly to Medina et al. (2012), from 9 = "excellent" to 1 = "extremely poor" and the limit of product marketability was considered 5 =

"Fair". When 30% of the treatment scored below 5, the experiment was terminated, marking the end of shelf life. All the overall visual quality ratings (OVQ) were discussed to reach a consensus between two expert judges. Random duplicated visual quality evaluations between judges were performed throughout the experiments to assure the veracity of the scores.

The surface color was measured on spinach leaves using an A5 Chroma-Meter Minolta CR-400 (Minolta Co. Ltd., Osaka, Japan). For each replication, the color of the adaxial surface of five leaves per treatment was measured in two spots that were diametrically opposed to the leaf axis. The color parameters were expressed with the CIEL*a*b* color system, L* is lightness and h° is hue angle, calculated as $\tan^{-1} b^*/a^*$ (McGuire, 1992).

Spinach leaf tenderness as resistance to shear force was measured using a texture analyzer TA-58, TA.XT.plus (Texture Technologies Corp., Scarsdale, NY, USA), with a 5-blade TA-91 Kramer Shear Cell (Texture Technologies Corp., Scarsdale, NY, USA). The return distance of the probe was set at 35 mm, the test speed at 1.7 mm/s, and the return speed at 10 mm/s. For each treatment, the midrib of 10 leaves per replication was removed and the leaves were weighed. The peak force required to shear the stack of 10 spinach leaves, was measured, similarly to Prakash et al. (2000). Leaf tenderness was calculated as the maximum force in Newton per gram.

Water content (WC) of the spinach leaves was measured using 5.0 grams of fresh leaf tissue that was removed from five randomly selected leaves per replication. The fresh leaf tissue (FW) samples were dried using an oven (Precision™, Thermo Fisher Scientific, Waltham, MA, USA) set at 80 °C. After 24 hours, the dry weight (DW) was measured. The water content was calculated by the equation WC (%)= [(FW-DW)/FW]x100 (Agüero et al., 2008).

The chlorophyll content of the spinach leaves was measured following the method of Wellburn (1994). For each treatment, 0.3 g of leaf tissue per replication was homogenized in 10 ml of methanol using a benchtop homogenizer (POLYTRON PT 1600 E, Kinematica Inc., Bohemia, NY, USA). The solution was incubated in darkness at 4 °C for 24 hours. Following the incubation period, an aliquot of the supernatant was measured at 653 nm and 666 nm in a 96-well microplate reader spectrophotometer (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA). Total chlorophyll content was calculated using the following equations: Chlorophyll a (Chl a): [15.65 x (Abs666) – (7.34 x (Abs653)]; Chlorophyll b (Chl b): [27.05 x (Abs653) – (11.21 x (Abs666)] and Total chlorophyll content = Chl a + Chl b. The total chlorophyll content was expressed as mg/100 g FW.

Nutritional Quality

The nutritional quality of spinach leaves was evaluated by measuring total phenolic content, antioxidant capacity, and vitamin C content. On each analysis day, spinach leaves were frozen using liquid nitrogen and stored at -20 °C for later analysis of the total phenolic content and antioxidant capacity. The frozen samples were freeze-dried using a Harvest Right Freeze drier (North Salt Lake, UT, USA). The freeze-dried samples were pulverized using a pestle and mortar. A solution of 20 ml Acetone/water (1:1) was used for extracting 0.2 g of freeze-dried spinach powder. The mixture was shaken at 80 rpm for 1 h using a 2314 Multi-Purpose Rotator (Thermo Fisher Scientific, Waltham, MA, USA) and then centrifuged (JA-17, Beckman Coulter, Palo Alto, CA, USA) at 12800 g at 4 °C for 20 minutes. Aliquots of the supernatant were analyzed for total phenolic content and antioxidant capacity. For the vitamin C measurements, at each day of the shelf life analysis, 2.0 g of spinach tissue from each experimental unit were homogenized with 20 ml of acid solution (6% metaphosphoric acid/ 2 N glacial acetic acid) using a benchtop homogenizer (POLYTRON PT 1600 E, Kinematica Inc., Bohemia, NY, USA) (Klimczak and Gliszczynska-wiglo, 2015). The Vitamin C samples were frozen at – 20 ˚C for later analysis.

The total antioxidant capacity was measured as the Ferric Reducing Ability of Plasma (FRAP) and the Oxygen Radical Absorbance Capacity (ORAC) of the spinach samples. FRAP was measured following the procedure of Benzie and Strain (1996). The extracted aliquots were analyzed in a 96-well microplate reader (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA) at 593nm absorbance. FRAP was expressed as micromolar Trolox equivalents on a 100 g FW basis (µmol TE/100 g FW).

ORAC was also analyzed spectrophotometrically following the method of Cao et al. (1993) and later modified by Ou et al. (2001) and Prior et al. (2003). ORAC was expressed as micromolar Trolox equivalent on a 100 g fresh FW basis (µmol TE/100 g FW).

The total phenolic content of the spinach samples was measured following the method of Singleton and Rossi (1965). The extracted aliquots were analyzed in a 96-well microplate reader (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA) at 750 nm absorbance. The total phenolic content was expressed in mg gallic acid equivalents on a 100 g FW basis (mg GAE/100g FW).

The Vitamin C content of the spinach samples was measured using Ultra Performance Liquid Chromatography (UPLC) with the method described by Klimczak and Gliszczynska-wiglo (2015). On the day of analysis, the frozen samples were thawed and centrifuged at 6177 g for 10 min (JA-17, Beckman Coulter, Palo Alto, CA, USA). The supernatant was further diluted with the acid solution (6% metaphosphoric acid/ 2N glacial acetic acid) and then filtered using a

0.2 µm PTFE syringe filter (VWR International, Radnor, PA, USA). A 5 µL aliquot of the diluted sample was injected in an Acquity Waters UPLC (Waters Corp., Milford, MA, USA) equipped with an Acquity BEH C18 column (Waters Corp., Milford, MA, USA). The flow rate was set at 0.2 mL/min and the linear gradient of the mobile phase was composed of 5 mM potassium phosphate monobasic (KH_2PO_4), pH 2.65 with 0.1% of formic acid (solution A) and methanol with 0.1% of formic acid (solution B). The linear gradient of the mobile phase was set at 5-15% B in 1 min, 15-35% B in the next 1 min and return to initial conditions in 4 min. Vitamin C was detected at 245 nm using the Acquity photodiode array detector (PDA) (Waters Corp., Milford, MA, USA). The samples were quantified using a five-point standard curve (2.5 µg/mL - 50 µg/mL) with purified ascorbic acid (assay percentage range ≥99.0 %, Fisher Scientific, Hampton, NH, USA) as a standard.

Statistical Analysis

All analyses were conducted using the statistical language R version 3.4 (R Foundation for Statistical Computing, Vienna, Austria). The equality of variance of all the data was investigated using Levene's tests and unequal variances were found ($P < 0.05$). The Welch one-way analysis of variance (ANOVA) was used instead of the traditional F-Test ANOVA, to account for the heterogeneous variances (Glass et al., 1977). The benefit of this analysis is that it is not affected by unequal sample size and is also robust against the absence of normality in the residuals of the models, as long as Pearson's moment coefficient of skewness is smaller than 2 (Lix et al., 2008). The dataset was evaluated for skewness and Pearson's moment coefficient was found to be smaller than 2 for the whole set.

The data from the two experimental years were combined and analyzed using Welch ANOVA with a Holm adjustment to account for unequal variances. Based on the presence of significant interactions between the treatment and year, the data was separated and analyzed per year. For evaluating differences ($P < 0.05$) in the measured quality parameters during shelf life, between the two production years, Welch ANOVA was used with a posthoc pairwise comparison using estimated marginal means with a Holm adjustment.

Results

Quality of spinach during storage at 3 °C

We observed significant interactions between years and production systems and years and storage days for the majority of the physical and nutritional quality parameters examined in this experiment (Table 3.1 P-values reflecting the effects of year (Y), production system (S), and storage day (D) and their interactions on respiration rate, overall visual quality (OVQ), leaf color, leaf tenderness, chlorophyll content, water content, total phenolic content, antioxidant capacity, and vitamin C content of spinach grown in high tunnel or open field from fall 2015 to spring 2017 and stored at 3 °C for 19 days.Table 3.1). For this reason, each year was analyzed and presented separately.

Table 3.1 P-values reflecting the effects of year (Y), production system (S), and storage day (D) and their interactions on respiration rate, overall visual quality (OVQ), leaf color, leaf tenderness, chlorophyll content, water content, total phenolic content, antioxidant capacity, and vitamin C content of spinach grown in high tunnel or open field from fall 2015 to spring 2017 and stored at 3 °C for 19 days.

Interactions[z]	**Respiration rate**[y]	**OVQ**	**Color hue**	**Color L**	**Leaf tender-ness**	**Chlorophyll content**	**Water content**	**Total phenolic content**	**FRAP**	**ORAC**	**Vitamin C content**
Year (Y)	<.001	<.001	<.05	<.001	<.001	NS[x]	<.001	<.001	<.001	<.001	NS[x]
Production system (S)	<.05	<.05	NS	<.05	<.001	NS	<.001	<.001	<.001	<.001	<.001
Storage day (D)	<.001	<.001	<.001	<.001	<.001	NS	NS	NS	<.001	<.001	<.001
Y x S	NS	NS	<.05	NS	<.05	<.05	<.01	<.05	<.01	NS	<.001
Y x D	<.001	<.001	NS	<.001	<.001	NS	NS	NS	NS	<.001	<.01
S x D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y x S x D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z] Welch ANOVA was used to test which factors and interactions between factors had significant effect on the examined quality parameters (α=0.05).

[y] The analysis included 2 harvests for the growing year 2015- 2016 and 2 harvests for production year 2016- 2017.

[x] NS = Non-significant

During the 2015-2016 production period (year 1), the respiration rate on the day of harvest of the spinach produced in the high tunnels was 1.2 times lower compared to the open field (*P* <.0001) (89.3 vs 110.0 mg CO_2/Kg-h) (Table 3.2). However, during storage, there was no difference in respiration rate between the two production systems (Table 3.2). The spinach grown in the high tunnels had similar visual quality as the spinach grown in the open field, with one exception. We observed significantly higher visual quality (*P* <0.01) after 9 days of storage for the spinach grown in the high tunnels when compared to spinach produce in the open field plots (7.2 vs 6.8) (Table 3.2). In year 1, there were no significant differences in leaf color between the spinach produced in the high tunnels and the open field plots (Table 3.2). Spinach grown in the high tunnels had significantly more tender leaves (*P* <0.01) after 9 days compared to the spinach grown in the open field plots (1.37 vs 1.62 N/g), but the leaf tenderness was similar between the production systems for the rest of the storage period (Table 3.2). The chlorophyll content of the spinach produced in the high tunnels was significantly higher (*P* <0.05) (114.2 vs 91.1 mg/100g FW) on the day of harvest than that of the spinach produced in the open field (Table 3.2), but no difference in chlorophyll content was observed between the two production systems during the storage life (Table 3.2). Spinach grown in the high tunnels had significantly higher water content (approximately 1.5%) than the spinach grown in the open field plots during the majority of the storage period, but there was no difference between the systems on the last day of shelf life, after 19 days of storage (Figure 3.1). During the 2015-2016 season, total phenolic content did not differ between high tunnel and open field systems during storage at 3 °C (Table 3.2). During the same production period, the antioxidant capacity (FRAP & ORAC) of the spinach grown in the high tunnels was significantly lower than in open field spinach on the day of harvest and throughout the storage life when stored at 3 °C (Table 3.3). Specifically, spinach grown in the high tunnels had 1.4 – 1.7 times lower FRAP and 1.2 – 1.6 times lower ORAC (Figure 3.2). There was no difference in vitamin C content between the two production systems on the day of harvest; however, high tunnel spinach had significantly lower vitamin C content throughout the rest of shelf life (Figure 3.3). Specifically, spinach grown in the high tunnels had 1.2 – 1.3 times lower vitamin C content during storage life than open field spinach (Figure 3.3).

Table 3.3 P-values reflecting the comparison of total phenolic content, antioxidant capacity and vitamin C content of spinach grown in high tunnel or open field in Olathe, KS, in 2015-2016 (Year 1) and 2016-2017 (Year 2) and stored at 3 °C for 19 days.

	Year 1[y]					
Measurement[z]	**Day 0**	**Day 5**	**Day 9**	**Day 13**	**Day 17**	**Day 19**
Total phenolic content[x]	NS[w]	NS	NS	NS	NS	NS
FRAP	<.001	<.001	<.001	<.001	<.001	<.001
ORAC	<.01	<.001	<.001	<.05	<.01	<.05
Vitamin C	NS	<.001	<.01	<.05	<.001	<.05
	Year 2					
Total phenolic content	.001	<.05	<.05	NS	<.01	<.001
FRAP	NS	NS	NS	NS	NS	NS
ORAC	NS	NS	NS	NS	<.05	NS
Vitamin C	NS	NS	NS	NS	NS	NS

[z] In all years, a "systems" experimental design was utilized with six replications per treatment; main treatment effect was high tunnel compared with open field production systems. Each replication represents a spinach plot consisting of an 8.5 m long bed with 5 rows each. Only mature leaves were harvested and immature leaves were left on the plant. The harvested spinach was stored at 3 °C for 19 days. The analysis included 2 harvests per production year.

[y] Data are separated by year due to significant interactions observed between the experimental factors.

[x] Welch Analysis of variance (ANOVA) was used to test the differences of the examined nutritional quality parameters between the two production systems($\alpha=0.05$).

[w] NS = Non-significant

Table 3.2 P-values reflecting the comparison of respiration rate, overall visual quality (OVQ), leaf color, leaf tenderness, chlorophyll content, and water content of spinach grown in high tunnel or open field in Olathe, KS, in 2015-2016 (Year 1) and 2016-2017 (Year 2) and stored at 3 °C for 19 days.

	Year 1[y]					
Measurement[z]	**Day 0[x]**	**Day 5**	**Day 9**	**Day 13**	**Day 17**	**Day 19**
Respiration rate[w]	<.001	NS[w]	NS	NS	NS	NS
OVQ	NS	NS	<.01	NS	NS	NS
Color L	NS	NS	NS	NS	NS	NS
Color Hue	NS	NS	NS	NS	NS	NS
Leaf tenderness	NS	NS	<0.05	NS	NS	NS
Chlorophyll content	<.05	NS	NS	NS	NS	NS
Water content	<.01	<.01	<.001	NS	<.05	NS
	Year 2					
Respiration rate	NS	NS	NS	NS	NS	NS
OVQ	NS	NS	NS	NS	NS	NS
Color L	NS	NS	NS	NS	NS	NS
Color Hue	NS	NS	NS	NS	NS	NS
Leaf tenderness	<.05	NS	NS	NS	<.05	<.01
Chlorophyll content	NS	NS	<.05	<0.01	NS	NS
Water content	<.001	<.001	<.01	<.001	<.001	<.001

[z] In all years, a "systems" experimental design was utilized with six replications per treatment; main treatment effect was high tunnel compared with open field production systems. Each replication represents a spinach plot consisting of an 8.5 m long bed with 5 rows each. Only mature leaves were harvested and immature leaves were left on the plant. The harvested spinach was stored at 3 °C for 19 days. The analysis included 2 harvests per production year.

[y] Data are separated by year due to significant interactions observed between the experimental factors.

[x] Welch analysis of variance (ANOVA) was used to test the differences of the examined physical quality parameters between the two production systems($\alpha=0.05$).

[w] NS = Non-significant

Figure 3.1 Water content of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2015-2016 (Year 1) and 2016-2017 (Year 2) and stored at 3 °C for 19 days. Each value represents the mean (±SD) of measurements obtained from two harvests.

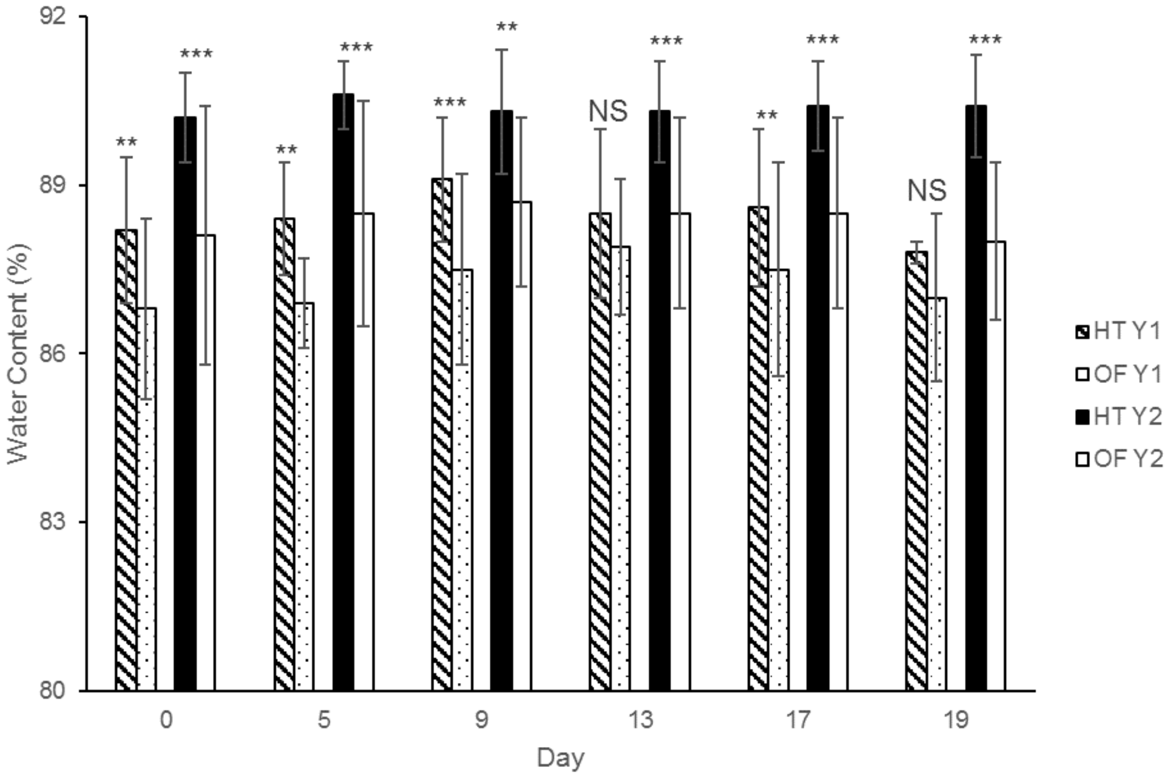


Table 3.3 P-values reflecting the comparison of total phenolic content, antioxidant capacity and vitamin C content of spinach grown in high tunnel or open field in Olathe, KS, in 2015-2016 (Year 1) and 2016-2017 (Year 2) and stored at 3 °C for 19 days.

	Year 1[y]					
Measurement[z]	**Day 0**	**Day 5**	**Day 9**	**Day 13**	**Day 17**	**Day 19**
Total phenolic content[x]	NS[w]	NS	NS	NS	NS	NS
FRAP	<.001	<.001	<.001	<.001	<.001	<.001
ORAC	<.01	<.001	<.001	<.05	<.01	<.05
Vitamin C	NS	<.001	<.01	<.05	<.001	<.05
	Year 2					
Total phenolic content	.001	<.05	<.05	NS	<.01	<.001
FRAP	NS	NS	NS	NS	NS	NS
ORAC	NS	NS	NS	NS	<.05	NS
Vitamin C	NS	NS	NS	NS	NS	NS

[z] In all years, a “systems” experimental design was utilized with six replications per treatment; main treatment effect was high tunnel compared with open field production systems. Each replication represents a spinach plot consisting

of an 8.5 m long bed with 5 rows each. Only mature leaves were harvested and immature leaves were left on the plant. The harvested spinach was stored at 3 °C for 19 days. The analysis included 2 harvests per production year.

[y] Data are separated by year due to significant interactions observed between the experimental factors.

[x] Welch Analysis of variance (ANOVA) was used to test the differences of the examined nutritional quality parameters between the two production systems(α=0.05).

[w]NS = Non-significant

Figure 3.2 Antioxidant capacity (ORAC and FRAP) of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2016-2017 (Year 1) and stored at 3 °C for 19 days. Each value represents the mean (±SD) of measurements obtained from two harvests.

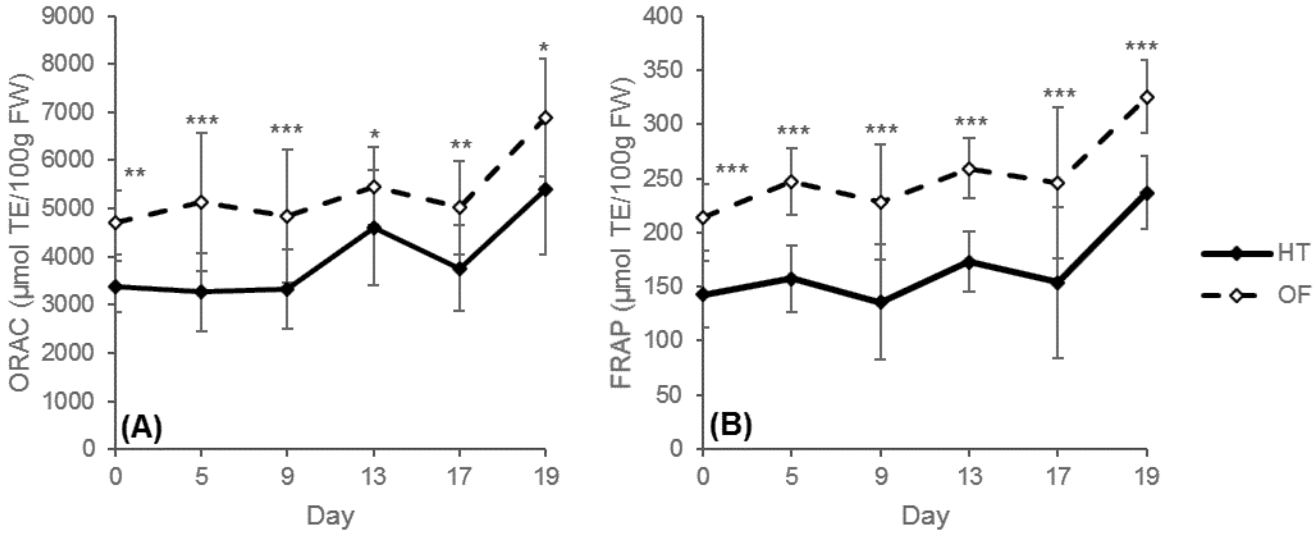
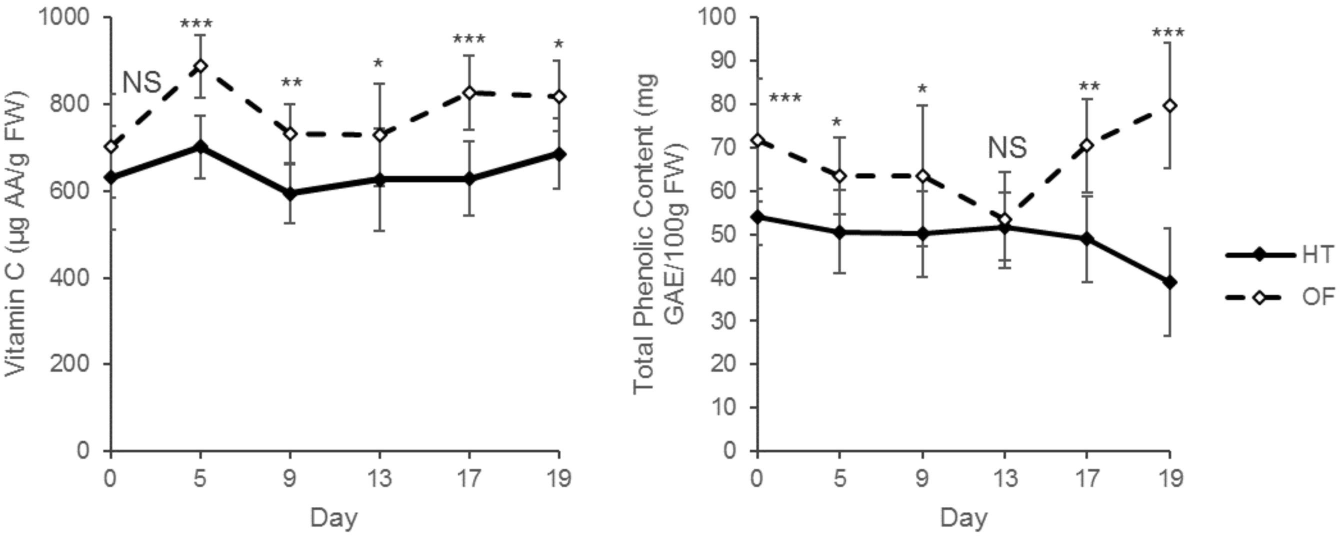


Figure 3.3 Vitamin C content of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2015-2016 (Year 1) and total phenolic content of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2016-2017 (Year 2) and stored at 3 °C for 19 days. Each value represents the mean (±SD) of measurements obtained from two harvests.



During the 2016-2017 production period (year 2), the respiration of spinach was similar for both growing systems at harvest and throughout the storage life (Table 3.2). We did not observe any significant differences in overall visual quality and leaf color between the two production systems at harvest and during storage for this production period (Table 3.2). Spinach grown in the high tunnels had leaves that were significantly more tender than open field spinach (1.3 vs 1.76 N/g, $P < 0.05$) on the day of harvest. No differences were observed in leaf tenderness during the majority of the storage period (Table 3.2) and only at the end of the shelf life when spinach grown in the high tunnels had significantly more tender leaves than open field spinach on day 17 (1.24 vs 1.73 N/g, $P < 0.05$) and day 19 (1.18 vs 1.86 N/g, $P < 0.01$). Regarding chlorophyll content, spinach produced in high tunnels had significantly higher chlorophyll content after 9 (102.1 vs 84.6 mg/100g FW, $P < 0.05$) and 13 days (117.8 vs 102.7 mg/100g FW, $P < 0.01$) of storage (Table 3.2) than the spinach grown in the open field plots. However, there was no significant difference in the chlorophyll content of the spinach produced in the two systems at the end of shelf life (Table 3.2). In year 2, the water content throughout storage of the spinach grown in the high tunnels was significantly higher (approximately 1.6% - 2.1%) than that of the spinach grown in the open field (Figure 3.1). In contrast to the first production year, during the second year there was no significant difference during storage life in the antioxidant capacity of the spinach produced in the two production systems (Table 3.3). However, spinach grown in the high tunnels had significantly lower phenolic content from the day of harvest to the end of shelf life than the spinach produced in the open field plots (Figure 3.3). Specifically, spinach grown in the high tunnels had 1.3 – 2 times lower phenolic content during storage than open field spinach (Figure 3.3). On the other hand, there were no significant differences in vitamin C content, between the two production systems (Table 3.3).

Quality of spinach stored at 13 °C

We observed significant interactions between year and system and year and storage day for the majority of the physical and nutritional quality (Table 3.4) parameters examined in this experiment. For this reason, each year was analyzed and examined separately

Table 3.4 P-values reflecting the effects of year (Y), production system (S) and storage day (D) and their interactions on respiration rate, overall visual quality (OVQ), leaf color, leaf tenderness, chlorophyll content, water content, total phenolic content, antioxidant capacity, and vitamin C content of spinach grown in high tunnel or open field from fall 2015 to spring 2017 and stored at 13 °C for 19 days.

Interactions[z]	**Respiration rate**[y]	**OVQ**	**Color hue**	**Color L**	**Leaf tender -ness**	**Chlorophyll content**	**Water content**	**Total phenolic content**	**FRAP**	**ORAC**	**Vitamin C content**
Year (Y)	<.05	<.001	NS[x]	<.001	<.001	<.001	<.001	<.001	<.001	NS	NS
Production system (S)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Storage day (D)	<.001	<.001	<.001	<.001	<.001	<.01	<.001	NS[x]	NS	<.001	<.001
Y x S	NS	NS	<.001	<.001	<.01	<.01	<.01	NS	<.001	NS	<.05
Y x D	NS	<.001	NS	NS	NS	<.01	NS	NS	<.05	<.001	<.01
S x D	NS	<.05	<.05	<.05	NS	NS	NS	NS	NS	NS	NS
Y x S x D	<.05	NS	NS	NS	<.05	<.05	NS	NS	NS	NS	NS

[z] Welch ANOVA was used to test which factors and interactions between factors had significant effect on the examined quality parameters (α=0.05).

[y] The analysis included 2 harvests for the production year 2015- 2016 and 2 harvests for production year 2016- 2017.

[x] NS = Non-significant

During the 2015-2016 production period (year 1), on the day of harvest, the spinach produced in high tunnels had 1.2 times lower respiration rate (89.3 vs 110 mg CO_2/Kg-h, *P* <.0001) than the spinach produced in the open field plots (Table 3.5). However, during storage, the respiration rate was similar for the two production systems (Table 3.5). The overall visual quality of spinach stored at 13 °C did not differ significantly between the two production systems during the majority of the storage life except on the last day of storage (Table 3.5). Specifically, after 9 days of storage the spinach produced in the high tunnels had significantly higher (*P* <0.01) overall visual quality than the spinach produced in the open field plots (5.8 vs 5.4) (Table 3.5). There were no differences in leaf color and tenderness, during storage at 13 °C between the spinach produced in open field plots and high tunnels. (Table 3.5). Spinach produced in the high tunnels had higher chlorophyll content (*P* <0.01) than open field spinach (114.2 vs 91 mg/100g FW) on the day of harvest in year 1, but there were no differences between the two production systems during the 9 days of storage (Table 3.5). Spinach grown in the high tunnels had significantly higher water content (approximately 1-1.4%) during most of the storage period than the spinach grown in the open field (Figure 3.4), but there was no difference between the two systems on the last day of shelf life (Figure 3.4). With regard to the nutritional quality of the spinach, during year 1 the spinach grown in the high tunnels had throughout storage, significantly lower antioxidant capacity in terms of FRAP and ORAC (Figure 3.5). Specifically, spinach grown in the high tunnels had 1.5-1.6 times lower FRAP and 1.2-1.4 times lower ORAC than open field spinach (Figure 3.5). There was no difference between the phenolic content of spinach produced in the high tunnels and open field plots during shelf life at 13 °C (Table 3.6). In year 1, vitamin C content did not differ between the two production systems on the day of harvest (631.5 vs 704.4 µg AA/g FW). However, after 9 days of storage at 13 °C, the high tunnel produced spinach had significantly lower ($P < 0.01$) vitamin C content than the open field produced spinach (458.3 vs 610.4 µg AA/g FW) (Table 3.6).

Table 3.5 P-values reflecting the comparison of respiration rate, overall visual quality (OVQ), leaf color, leaf tenderness, chlorophyll content, and water content of spinach grown in high tunnel and open field in Olathe, KS, in 2015-2016 (Year 1) and 2016-2017 (Year 2) and stored at 13 °C for 9 days.

	Year 1[y]				
Measurement[z]	**Day 0**	**Day 3**	**Day 5**	**Day 7**	**Day 9**
Respiration[x] rate	<.01	NS[x]	NS	NS	NS
OVQ	NS[w]	NS	NS	NS	<.01
Color L	NS	NS	NS	NS	NS
Color hue	NS	NS	NS	NS	NS
Leaf tenderness	NS	NS	NS	NS	NS
Chlorophyll content	<.01	NS	NS	NS	NS
Water content	<.01	NS	<.05	<.05	0.0584
	Year 2				
Respiration rate	NS	<.05	<.05	<.05	<.05
OVQ	NS	NS	NS	<.01	0.0515
Color L	NS	<.05	<.01	<.001	<.001
Color hue	NS	NS	<.05	<.01	<.05
Leaf tenderness	<.001	<.001	NS	NS	NS
Chlorophyll content	NS	NS	<.001	NS	<.001
Water content	<.001	<.001	<.001	<.001	<.05

[z] In all years, a "systems" experimental design was utilized with six replications per treatment; main treatment effect was high tunnel compared to open field production systems. Each replication represents a spinach plot consisting of an 8.5 m long bed with 5 rows each. Only mature leaves were harvested and immature leaves were left on the plant. The harvested spinach was stored at 3 °C for 19 days. The analysis included 2 harvests per production season.

[y] Data are separated by year due to significant interactions observed between the experimental factors.

[x] Welch analysis of variance (ANOVA) was used to test the differences of the examined physical quality parameters between the two production systems ($\alpha=0.05$).

[w] NS = Non-significant

Figure 3.4 Water content of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2015-2016 (Year 1) and 2016-2017 (Year 2) and stored at 13 °C for 9 days. Each value represents the mean (±SD) of measurements obtained from two harvests.

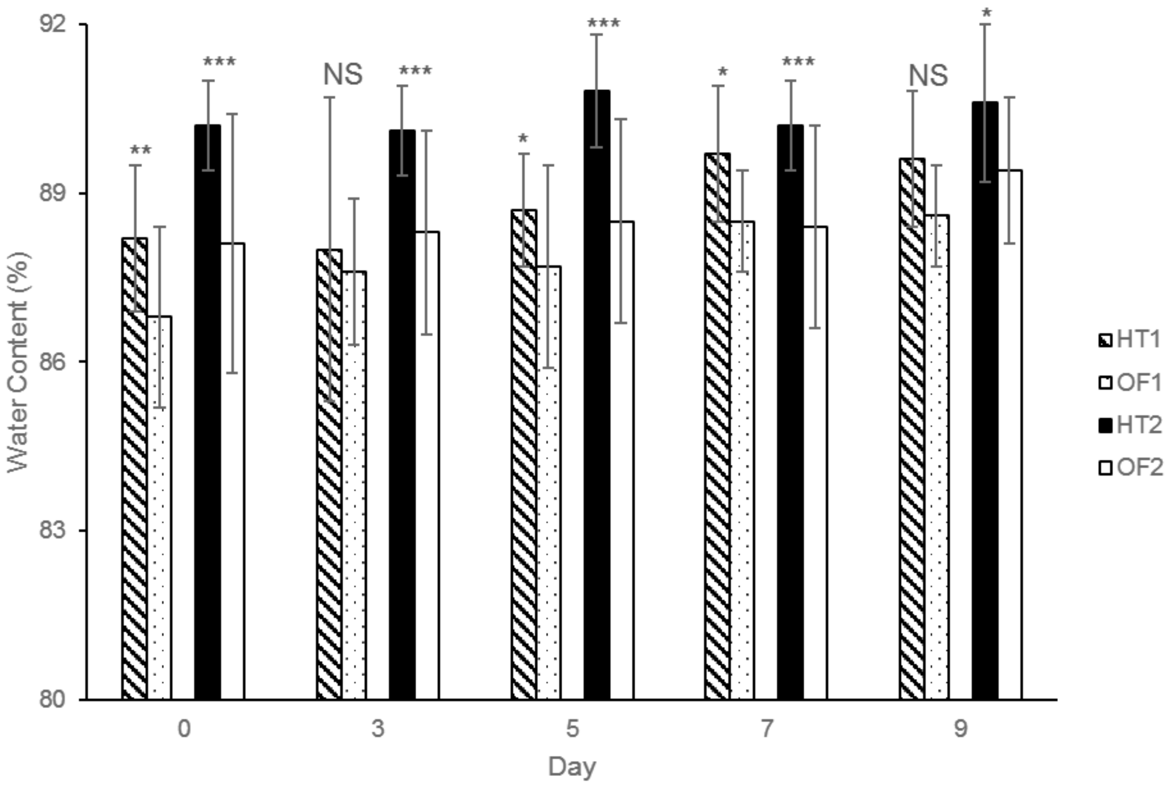


Figure 3.5 Antioxidant capacity (ORAC and FRAP) of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, 2016-2017 (Year 1) and stored at 13 °C for 9 days. Each value represents the mean (±SD) of measurements obtained from two harvests.

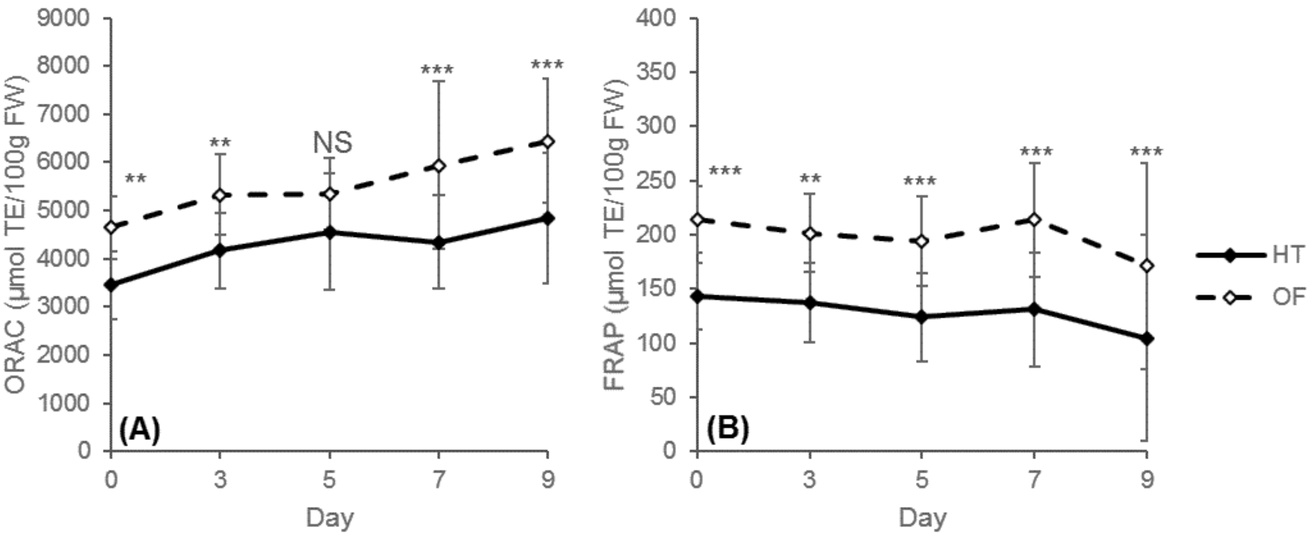


Table 3.6 P-values reflecting the comparison of total phenolic content, antioxidant capacity, and vitamin C content of spinach grown in high tunnel or open field in Olathe, KS, in 2015-2016 (Year 1) and in 2016-2017 (Year 2) and stored at 13 °C for 9 days. Each value represents the mean of measurements obtained from 2 harvests, one in fall and one in spring.

	Year 1^{y}				
Measurement^{z}	**Day 0**	**Day 3**	**Day 5**	**Day 7**	**Day 9**
Total phenolic content^{x}	NS^{w}	NS	NS	NS	NS
FRAP	<.001	<.01	<.001	<.001	<.001
ORAC	<.01	<.01	NS	<.001	<.001
Vitamin C content	NS	<.01	NS	NS	<.01
	Year 2				
Total phenolic content	<.01	NS^{x}	NS	<0.001	NS
FRAP	NS	NS	NS	NS	NS
ORAC	<.05	NS	NS	<.05	<.01
Vitamin C content	NS	NS	NS	NS	NS

^{z}In all years, a "systems" experimental design was utilized with six replications per treatment; main treatment effect was high tunnel compared to open field production systems. Each replication represents a spinach plot consisting of an 8.5 m long bed with 5 rows each. Only mature leaves were harvested and immature leaves were left on the plant. The harvested spinach was stored at 3 °C for 19 days. The analysis included 2 harvests per production year

^{y} Data are separated by year due to significant interactions observed between the experimental factors.

^{x} Welch analysis of variance (ANOVA) was used to test the differences of the examined nutritional quality parameters between the two production systems (α=0.05).

^{w}NS = Non-significant

During the 2016-2017 production period (year 2), while the respiration rate did not differ between the two production systems on the day of harvest, spinach produced in the high tunnels had consistently 1.4-1.5 times lower ($P < 0.05$) respiration rate than open field spinach during storage at 13 °C (Figure 3.6). In year 2, after 7 days of storage spinach produced in the high tunnels had significantly higher ($P < 0.01$) overall visual quality than spinach produced in the open field (5.8 vs 5.1) (Table 3.5). While on the last day of storage the high tunnel spinach demonstrated higher overall visual quality compared to the open field spinach (4.8 vs 4.3), the difference was not significant ($P = 0.051$) (Table 3.5). After 3 days of storage until the end of storage life the spinach produced in the high tunnels had consistently significantly darker leaves than the spinach produced in the open field plots (Figure 3.7). In addition, the spinach produced in the high tunnels had significantly higher hue values after 5 days of storage at 13 °C, until the end of the shelf life (Figure 3.7). The spinach produced in the high tunnels had more tender ($P < 0.001$) leaves on the day of harvest up to the fifth day (1.3 vs 1.75 N/g) of storage at 13 °C, but for the rest of the shelf life the leaf tenderness was similar for spinach from the two production systems (Table 3.5). In the second year of the experiment, there were no differences in chlorophyll content on the day of harvest between the two production systems (101.1 vs 93.3 mg/100g FW) (Table 3.5). However, the spinach produced in the high tunnels had 1.4 times higher ($P < 0.001$) chlorophyll content after 9 days of storage than the open field spinach (129.5 vs 91.5 mg/100g FW) (Table 3.5). Spinach grown in the high tunnels had significantly higher water content throughout storage (approximately 1.2%-2.3%) than the spinach grown in the open field plots (Figure 3.4). With regard to the nutritional quality, in year 2 there were no differences in the FRAP values between the two production systems (Table 3.6). However, spinach produced in the high tunnels had 1.2 times lower ($P < 0.05$) ORAC value on the day of harvest than open field spinach (Figure 3.6). On days 3 and 5 of storage, there were no differences in ORAC values between the two production systems, but at the end of shelf life high tunnel spinach had significantly lower (*P* <0.01) ORAC values than the open field spinach (1.3 times lower) (Figure 3.6). In the second year of the experiment, there were no differences in total phenolic content and vitamin C content between the two production systems (Table 6).

Figure 3.6 Respiration rate and antioxidant capacity (ORAC) of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2016-2017 (Year 2) and stored at 13 °C for 9 days. Each value represents the mean (±SD) of measurements obtained from two harvests.

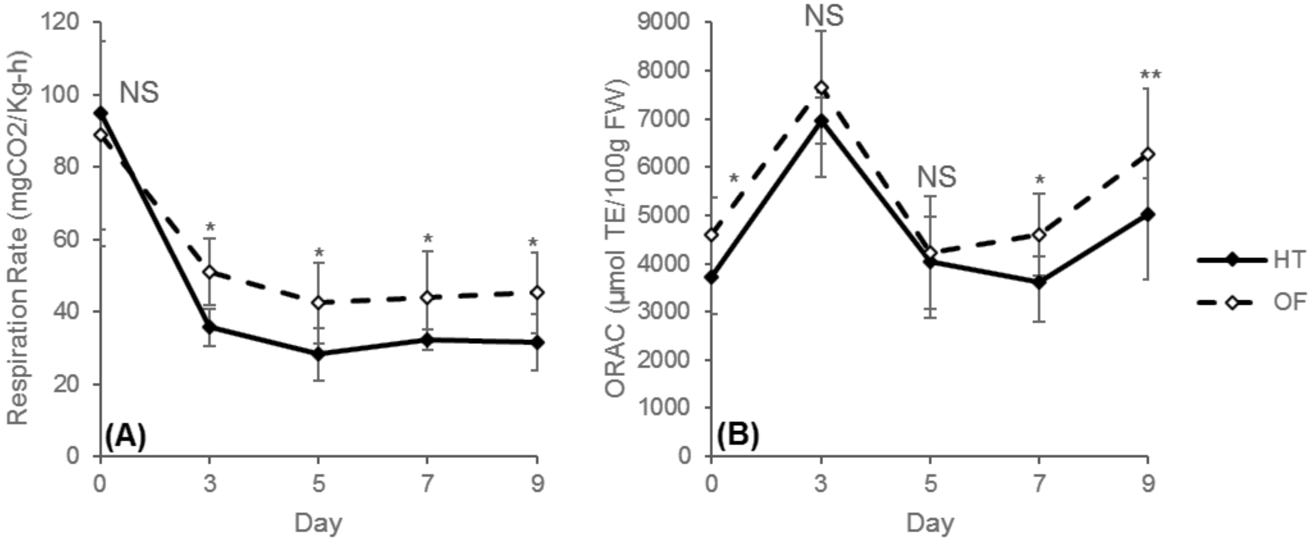
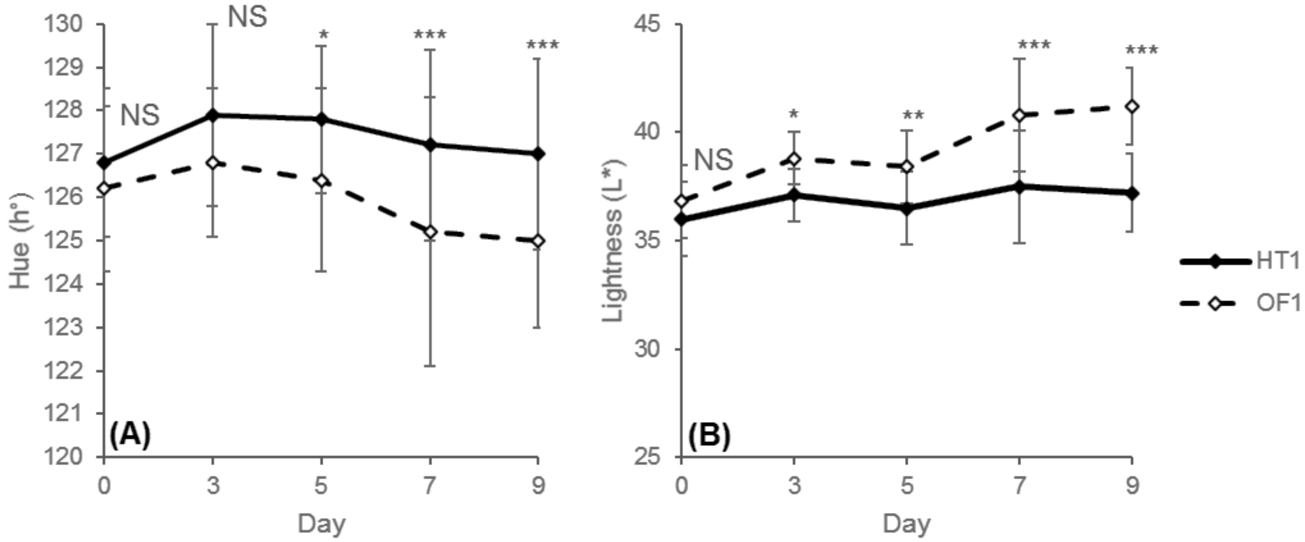


Figure 3.7 Leaf color (Hue and Lightness) of spinach grown in high tunnel (HT) or open field (OF) in Olathe, KS, in 2016-2017 (Year 2) and stored at 13 °C for 9 days. Each value represents the mean (±SD) of measurements obtained from two harvests.



Discussion

The goal of this study was to examine the effect of high tunnel and open field production systems in combination with optimum and non-optimum storage temperatures, on the postharvest food losses of spinach during storage including the physical, organoleptic, and nutritional quality of spinach.

Lack of or poor temperature control is one of the main factors contributing to food losses of fresh fruits and vegetables in the postharvest chain (Jedermann et al., 2014). Temperature is the most important factor affecting the postharvest quality and shelf life of fruits and vegetables (Saltveit, 2003; Prusky, 2011; Kader, 2013). This is directly linked to the fact that temperature dictates the rates of many of the metabolic processes and physiological responses occurring in the plant tissues during storage (Sams, 1999; Kader and Saltveit, 2003). These include respiration (Kader and Saltveit, 2003; Kitinoja and Kader, 2003), ethylene sensitivity (Brecht, 1995; Kitinoja and Kader, 2003), compositional changes (Prusky, 2011), water loss (Kitinoja and Kader, 2003; Prusky, 2011), and physiological breakdown (Brecht, 1995; Kader, 2013). As a result, non-optimum storage temperature has a detrimental effect on the quality and shelf life of fresh produce (Bartz and Brecht, 2002; Kader, 2013; Vicente et al., 2014). Our results are in accordance with this theorem. As anticipated, the spinach stored at 3 °C better retained its quality characteristics during storage, resulting in a longer shelf life compared with spinach stored at 13 °C. However, when observing the quality characteristics of the spinach stored at these two temperatures, there were minimal physical quality differences between the spinach produced in high tunnel and open field during shelf life at 3 °C. However, at 13 °C, the spinach from the examined production systems demonstrated differences in its postharvest behavior. In both years of the shelf life experiment at 13 °C, the spinach produced in the high tunnels had higher overall visual quality during storage and, in one of the two examined years, it also had lower respiration and decreased rate of chlorophyll breakdown throughout storage than open field spinach. These results indicate that the high tunnel growing system may contribute to reduction of the postharvest losses of spinach caused by temperature abuse.

Preharvest abiotic stress can increase the postharvest deterioration susceptibility of a commodity (Toivonen and Hodges, 2011). Abiotic stressors during growth in the field include, water, temperature, and light stress, among others (Joyce et al., 1998). Generally, when a crop is subjected to severe environmental stress during growth and particularly close to the period of harvest or/and during harvest, there is a detrimental effect on its postharvest quality due to phenomena like increased respiration, water loss, decay, and increased susceptibility to enzymatic browning during storage (Fonseca, 2006; Luna et al., 2013). The negative effect of these preharvest stressors is not always visible during harvest, but becomes evident during postharvest storage. This phenomenon has been described as latent damage (Hung, 1993). The high tunnel production system creates a protective microclimate that can reduce the effect of abiotic stressors such as strong winds, heavy rain, and hail (Lamont, 2005; Giacomelli, 2009; Zhao and Carey, 2009). In this study, we did not collect microclimate data from the two growing systems, but excessive rainfall (Hoppenstedt et al., 2019) and strong winds (Lu et al., 2009; Zhao and Carey, 2009) are common challenges for open field production in the Central U.S. Prolonged exposure to these stressors can influence the reactive oxygen species (ROS) levels in the plant tissue and the tissue ROS scavenging capacity, resulting in higher postharvest oxidative stress (Hodges et al., 2004; Bonasia et al., 2019) and an increased rate of compositional change during storage (Bonasia et al., 2019). Optimal storage temperature typically minimizes the oxidative stress while storage at above optimum temperature increases the oxidative stress and senescence rate of plant tissue (Toivonen, 2003). The effect of latent damage on product quality is affected by postharvest exposure to increased temperature (Romig, 1995). This could explain the observed differences between the spinach from the two production systems stored at 13 °C. The spinach grown in the protected microclimate created by the high tunnel is likely subjected to less abiotic stress during growth than spinach grown in open fields, which possibly resulted in higher tissue scavenging capacity in the high tunnel spinach. Thus, when the spinach produced in these two production systems is stored in a non-optimum temperature, the latent stress effect could be exacerbated in the open field spinach. For the spinach stored at 3 °C, on the day of the harvest the spinach produced in the high tunnels exhibited characteristics indicating reduced stress such as increased water content and reduced respiration rate. Such characteristics of leafy greens are indicative of potential for longer shelf life (Gil, 2016). However, we found no difference in shelf life between the two systems during storage at 3 °C, which aligns with the fact that optimum temperature is the most effective postharvest tool for reducing losses. Generally, pre-harvest environmental conditions affect various plant physiological processes, like energy metabolism and water and nutrient uptake, and these processes are tied with the postharvest behavior of the crop (Beverly et al., 1993). While examining these processes in depth was outside the scope of this project, future research should examine the effect of high tunnels on the physiological and metabolic processes of spinach. Understanding how these processes are influenced by the high tunnel production system may allow further improvement of the postharvest behavior of commodities produced in this system.

The effect of preharvest factors, such as genotype, environmental conditions, microclimate, and cultural practices, on postharvest quality of fresh fruits and vegetables has been well-reported (Weston and Barth, 1997; Mattheis and Fellman, 1999; Sams, 1999; Kader, 2000; Toivonen and DeEll, 2002). These factors have been recognized to have a significant effect on the postharvest quality and shelf life of leafy greens (Weston and Barth, 1997; Gil, 2016). For instance, fresh-cut ‘lollo rosso' and ‘red oak leaf' lettuce grown in a soilless production system demonstrated a slower rate of deterioration and longer shelf life compared with the same lettuce cultivars grown in a system utilizing soil (Selma et al., 2012). In regards to spinach, (Johnson et al., 1989) reported that excessive rainfall during growth resulted in a 40% reduction of the shelf life potential of the crop. Similarly, application of the fungicide azoxystrobin on spinach during growth resulted in reduced water loss during storage (Conversa et al., 2014). In our study, the spinach produced in the high tunnels demonstrated consistently higher water content on the day of harvest. Wind exposure and wind speed can increase evapotranspiration in plants (Foroud et al., 2010; Falamarzi et al., 2014). The spinach grown in the high tunnel was protected from the wind, which may explain the increased water content compared with the spinach produced in the open field. We also observed lower respiration rate at harvest for the spinach produced in the high tunnel in the first year of the experiment. Plant respiration is affected by the surrounding plant environment and particularly by abiotic stress such as water stress, ultraviolet (UV) irradiation, and temperature extremes (Plaxton and Podestá, 2006). The protective microclimate created in the high tunnel and the fact that its polyethylene cover typically blocks UV light (Costa et al., 2003) might account for the decreased respiration rate at harvest. Additionally, the spinach produced in the high tunnel demonstrated higher chlorophyll content and tenderer leaves on the day of harvest. UV light exposure has been related to increased leaf thickness and decreased chlorophyll content (Huché-Thélier et al., 2016).

The postharvest losses of leafy greens are primarily defined by the rate of water loss and chlorophyll breakdown induced yellowing during storage (Cantwell and Kasmire, 2002). Our results indicate that the high tunnel production system can reduce the postharvest food losses of spinach when stored in non-optimum temperature, as evaluated by these parameters. While we did not measure water loss in this study, high tunnel spinach demonstrated consistently higher water content during storage. Water content in leafy vegetables is directly related to their textural properties (Sams, 1999). However, the spinach grown in the two production systems did not demonstrate many differences in leaf tenderness, during storage at both temperatures. High tunnel spinach in the second year had more tender leaves towards the end of shelf life at 3 °C. This difference between the production systems with regard to water content and tenderness might be attributed to the fact that the open field produced thicker spinach leaves while the high tunnel produced leaves with thinner cell walls consistent with higher water content, which implies lower dry matter content (Batziakas et al., 2019a). The combination of higher water content with a slower rate of yellowing when stored at 13 °C resulted in higher overall visual quality and longer shelf life for high tunnel spinach compared with the spinach produced in the open field.

Light intensity and light quality during growth affects the quality of leafy greens (Weston and Barth, 1997; Gil, 2016) and particularly the leaf pigmentation. It has been reported that in high tunnels using polyethylene film the diffuse PAR can reach 150% to 200% higher values than in the open field (Retamal-Salgado et al., 2015). Plants utilize diffuse PAR more efficiently than direct PAR (Gu et al., 2002) and it has been shown that diffuse PAR increases the photosynthesis and growth of crops grown in a protected environment (Li et al., 2014). Increased photosynthesis is correlated with increased chlorophyll content (Liu et al., 1984; Dodd et al., 2005; Ahammed et al., 2012). Additionally, the polyethylene film used in high tunnels blocks UV light (Costa et al., 2003; Espí et al., 2006). UV-B light can reduce the photosynthetic activity of spinach (Iwanzik et al., 1983) and decrease chlorophyll content (Huché-Thélier et al., 2016). This may explain the increased chlorophyll content of spinach produced in the high tunnels compared with the open field spinach. Chlorophyll breakdown is a response to a variety of abiotic stressors (Hörtensteiner and Kräutler, 2011) such as drought or excessive rainfall (De Luca d'Oro, G. M., & Trippi, 1987), heat and salinity (Khanna-Chopra, 2012). These stressors induce oxidative stress and initiate senescence (Hodges et al., 2004), with one of its symptoms being pigment degradation (Yamauchi and Watada, 1991; Fan et al., 2014).

In spinach, leaf chlorophyll breakdown and color change have been directly related to ROS regulated senescence (Yamauchi and Watada, 1991). Increased storage temperature accelerates senescence and chlorophyll breakdown in spinach (Pandrangi and LaBorde, 2004). At 13 °C, the spinach produced in the high tunnels had lower respiration rate during storage, which indicates a slower senescence rate compared with the open field produced spinach. This relationship explains the similar behavior of the spinach grown in the two systems with regard to chlorophyll and leaf color when stored at 3 °C and the better chlorophyll and color retention of the high tunnel spinach when stored at 13 °C. The difference in chlorophyll breakdown between the two production systems during shelf life at 13°C was more evident when examining leaf hue and lightness than leaf chlorophyll content. This result is similar to what Bergquist et al., (2006) and Fan et al., (2014) reported for spinach. This discrepancy between leaf color measurements and chlorophyll content might be related to the uneven and patchy chlorophyll loss in spinach leaves (Bergquist et al., 2006). Moreover, the colorimeter measures only small areas of the crop (an 8-mm diameter circle) while the chlorophyll analysis involves a more homogenous mixture of plant tissue (Proulx et al., 2010).

Postharvest food losses do not only involve physical quality characteristics that are visible to the consumer but also include the deterioration of nutritional quality (Kader, 2005). Spinach grown in the high tunnels consistently demonstrated significantly lower antioxidant capacity measured as ORAC and, in the first year of the experiment, lower FRAP and total phenolic content compared to spinach grown in the open field, on the day of harvest. Similar to our results, Zhao et al. (2007) reported that high tunnel spinach had lower ORAC values than open field spinach, and high tunnel lettuce had lower total phenolic content compared to the open field (Zhao et al., 2007c). In vegetables, increased antioxidant capacity and phenolic content have been linked with the response of the secondary metabolism to exposure to abiotic stressors such as UV light, water stress, and extreme temperature (Cisneros-Zevallos, 2003). The observed variation in physical and nutritional quality between the 2 years of this experiment can be attributed to the variation of climatic and environmental conditions between the two growing periods (Shewfelt, 1990; Tudela et al., 2013). During shelf life, the spinach produced in the high tunnels had lower nutritional quality compared to open field spinach as indicated by total phenolic content, antioxidant capacity, and vitamin C content.

In rocket salad, the antioxidant activity of the leaves has been shown to be affected by the production system, and increased antioxidant activity was related to improved postharvest performance and reduced decay (Bonasia et al., 2019). Similarly, in spinach, increased vitamin C at harvest has been linked with improved visual quality (Bergquist et al., 2006) and a decreased rate of vitamin C degradation is linked to slower senescence rate (Hodges and Forney, 2003) during storage. However, the behavior of antioxidant capacity is not always correlated with postharvest behavior of fresh produce and quality loss is not necessarily associated with a reduction of antioxidant concentration (Hodges and DeLong, 2007). In our experiment, while spinach produced in the high tunnels demonstrated reduced ROS scavenging ability as demonstrated by its phenolic content, antioxidant capacity, and vitamin C, it retained its quality for longer or it had similar quality as the open field spinach. The postharvest quality of fresh produce involves a synergy of various parameters and is not reflected just through antioxidant metabolism (Hodges and DeLong, 2007). Quality involves physical, organoleptic and nutritional attributes and postharvest behavior is affected by a multitude of factors (Kader, 2000). It should also be noted that high tunnel spinach demonstrated lower nutritional quality attributes does not necessarily mean that it is a product of inferior quality. The total phenolic content (Chun et al., 2005; Asfi et al., 2012), ORAC (Haytowitz and Bhagwat, 2010), FRAP (Machado et al., 2018), and vitamin C (Gil et al., 1999; Bergquist et al., 2006) values, were still within ranges that have been previously reported for spinach.

Recent reports highlight further the ability of high tunnels to reduce food losses. High tunnel spinach production reduces the pre-harvest losses of this crop, as the result of increased productivity, marketability, and premium crop quality (Batziakas et al., 2019a). Consumer dissatisfaction is a factor contributing to postharvest food waste (Baldwin, 2002; Kader, 2005) In a blind consumer study, the spinach produced in high tunnels was preferred compared to spinach grown in the open field in terms of overall liking, flavor, and texture (Batziakas et al., 2019b). All the above indicate that high tunnels are a growing system that can be utilized for meeting global food demand and should be incorporated in resilient food systems.

Conclusions

The results of this study show that production of spinach in high tunnels can reduce the postharvest FLW of this crop when stored in non-optimum temperatures, particularly as they relate to its physical and organoleptic quality. To our knowledge, this is the first study to report the effects of high tunnel production on the quality of spinach during postharvest storage. The spinach produced in both production systems was of superior quality as indicated by the physical and nutritional quality attributes measured on the day of harvest. There were no major differences between the spinach produced in the two production systems during storage at 3 °C. However, the spinach produced in the high tunnels exhibited higher quality and longer shelf life when stored at 13 °C, as indicated by reduced respiration rate, decreased yellowing rate, and higher water content. These results highlight the fact that temperature control is the most effective postharvest tool for maintaining the quality and extending the shelf life of fresh produce. Further research is needed for investigating the effect of the high tunnel production system on the crop and stress physiology of fresh produce for improving further the efficiency of this production system.

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Chapter 4 - Descriptive analysis and consumer acceptability of locally and commercially-grown spinach

Abstract

The consumer demand for locally grown fresh produce is continuously increasing in the United States. The high tunnel systems have been successfully utilized by small acreage growers for local production. Consumers are typically assessing appearance, freshness, flavor and aroma when purchasing produce. A common perception is that locally grown produce tastes better than non-local. However, there is not much evidence for supporting this claim. The objective of this study was to identify consumer acceptability and the sensory characteristics/differences of locally grown spinach in open field or in high tunnel and non-local commercially grown spinach. Spinach, Spinacia oleracea cv. "Corvair" was grown in open field and in high tunnel at Kansas State University Olathe Horticulture Research and Extension Center (OHREC) in spring 2017 and the commercially grown spinach was purchased at a local retail store. A consumer study (n=205) was conducted at Kansas State University, Olathe campus, and a descriptive sensory analysis was conducted by a highly trained descriptive analysis panel in the Center for Sensory Analysis and Consumer Behavior at Kansas State University, Manhattan campus, in spring 2017. The consumer test showed that high tunnel spinach scored significantly higher in overall liking ($p<0.0001$), flavor liking ($p<0.0001$) and texture liking ($p<0.05$) when compared to open field and store purchased spinach. Descriptive analysis showed that locally grown spinach had higher intensity of attributes that indicate premium quality, such as green color and green/spinach flavors. Our results indicate that locally grown spinach was preferred from the consumers for its high organoleptic quality

Introduction

Local food is generally described as food that does not travel a long distance from production to retail and/or is sold directly by the producer to the consumer (Watts et al., 2005). Consumption of local food in the U.S has been steadily increasing over the years (Nie and Zepeda, 2011; Zumkehr and Campbell, 2015). Local food sales in the U.S increased more than 140% from 2008 to 2014, reaching $12 billion, and is estimated to reach $20 billion by 2019 (U.S. Department Of Agriculture., 2016). The drivers for the increase in demand and consumption of local food are numerous and of diverse nature. They include food provenance concerns, trust relationship between consumers and producers (Marsden et al., 2000), human connection (Hinrichs, 2000), interest in sustainable agriculture/environmental concerns (Hinrichs, 2000; Brown, 2003; Brown et al., 2009; Adams and Adams, 2011), transparency (Feldmann and Hamm, 2015), taste (Adams and Adams, 2011; Grebitus et al., 2013), quality (Carpio and Isengildina-Massa, 2009) farmer/farmworker welfare (Carpio and Isengildina-Massa, 2009; Adams and Adams, 2011) and support of the local economy (Carpio and Isengildina-Massa, 2009; Grebitus et al., 2013). Several studies have reported that consumers associate some of the latter attributes to locally-produced food and are willing to pay a premium price for those products (Carpio and Isengildina-Massa, 2009; Adams and Adams, 2011; Onozaka and McFadden, 2011; Grebitus et al., 2013; Campbell et al., 2014; Gracia et al., 2014). Local food demand has also been closely connected to the demand for organic food. Consumers of locally-produced food value organic production methods (Conner et al., 2009) and organic food consumers value local food production (Gracia et al., 2014; Hempel and Hamm, 2016).

Local food production is frequently related with low-input, sustainable and/or organic production methods (Morgan and Murdoch, 2000; Seyfang, 2006; Macias, 2008). A local produce grower's survey in Kansas City metropolitan area showed that 86% of the respondents utilize organic growing methods and 63% are using sustainable growing methods (Greater Kansas City Food Hub Working Group, 2015). High tunnels are similar to an unheated greenhouse covered with a polyethylene sheet and crops are generally grown in the ground/soil. High tunnels are frequently utilized in local vegetable production throughout the U.S. (Carey et al., 2009) and have been adopted by many local vegetable growers in the Central U.S. (Greater Kansas City Food Hub Working Group, 2015). This growing system has been successfully used for protection from the weather elements, (Lang, 2009), increasing yield (Waterer, 2003; Lamont, 2005), increasing product quality (Lamont, 2005; Rogers and Wszelaki, 2012), season extension (Borrelli et al., 2013; Galinato and Miles, 2015; Gude et al., 2018) and reducing crop disease (Lang, 2009; Rogers and Wszelaki, 2012; Hanson et al., 2016) of fresh fruits and vegetables. Spinach, *Spinacia oleracea*, is a cold season crop frequently grown in high tunnels in the U.S. Great Plains Area (Knewtson et al., 2010). The season extension ability of high tunnels in combination with the cold hardiness of spinach can provide production during winter dormancy periods whereas this may not be feasible in the open-field. High tunnel spinach production has been found to decrease the antioxidant content of spinach (Zhao et al., 2007c) but the effect of this growing system on the quality and organoleptic characteristics of spinach is unknown.

Taste, freshness and appearance are important attributes affecting consumer food choices (Weatherell et al., 2003). For consumers of locally-produced fresh produce, the most important attributes are taste, freshness and quality (Selfa and Qazi, 2005). The most common perception amongst local food consumers is that locally produced food has better taste (Feldmann and Hamm, 2015) and is of higher quality (Brown, 2003; Feldmann and Hamm, 2015) when compared to food outside the region. One report indicated that consumers are relating freshness and better taste to shorter transportation distance (Roininen et al., 2006). (Costanigro et al., 2014) proposed that local food consumers have a positive bias towards local agriculture. This bias can affect the consumer acceptability and perception of sensory attributes of a product. Local apple juice was liked more by consumers when they were knowledgeable of its origin compared to blind tasting (Stolzenbach et al., 2013). In contrast, locality did not have a consistent effect on consumer preference during a blind consumer test with apples (Costanigro et al., 2014). Although these studies provide some insight into consumer trends, leafy greens have a much shorter shelf life and potential for quality loss than fruit crops like apple. The course of the produce through the supply chain may affect its quality characteristics (Edwards-Jones et al., 2008) with adverse effects on quality and flavor (Kader, 2000), which can result in deleterious effects on taste and other sensory characteristics. However, there is little information about the effect of the locality on the consumer acceptability and sensory characteristics of fresh produce. The objective of this study is to identify consumer acceptability and the sensory characteristics of spinach grown locally in the Central U.S., in open-field and high tunnel production systems as well as non-local purchased (commercially grown) spinach that was grown in Salinas California, using a blind consumer test and descriptive sensory analysis.

Materials and Methods

Samples

This research project tested spinach (*Spinacia oleracea)* grown in three different production systems. Local spinach grown in high tunnels and open-field plots, and commercially-grown, spinach that was purchased from a local retail store. Local spinach (*Spinacia oleracea* cv. "Corvair") was grown using standard organic growing practices for the region (Buller et al., 2016), at the K-State Olathe Horticulture Research and Extension Center (OHREC), located in Olathe, Kansas USA. Spinach seeds were germinated and grown in 72 cell trays, in an unheated greenhouse, until the development of two to four true leaves. Prior to transplanting, the seedlings were fertilized with Organic Neptune's Harvest (Neptune's Harvest, Gloucester, MA, U.S.A.) at the recommended rate (30 ml per 3785 ml of water). No fertilizer was applied to the beds before or after transplanting the seedlings. The seedlings were transplanted in the experimental plots during the fall 2016 season and were overwintered to spring of 2017. The plots consisted of six high tunnels (Quonset style with 1.5m sidewalls) (Stuppy, North Kansas City, MO., U.S.A.) and six open-field plots (9.8 m × 6.1 m) adjacent to the tunnels. The tunnels consisted of 10-mm twin- wall polycarbonate end walls and a single layer 0.15 mm polyethylene roof and sidewalls. The soil consisted of Chase silt loam (pH= 6.3). The spinach plot consisted of two beds, 91.5 cm apart from each other (center-to-center), with 4 rows each. The distance between rows was 30 cm and the spinach was planted at 15 cm in-row spacing. A 91.5 cm buffer zone was left unplanted on either end of the row. The locally grown spinach was harvested three days prior to the consumer test and five days prior the descriptive analysis. It was washed three times in ice-cold tap-water. Next, the spinach was dried with a manual 5-gallon salad spinner and stored in odorless produce bags at 0.5°C and 95% RH until the day of the study. The commercially-grown spinach, Muzzi Family Farms Organic Baby Spinach (Muzzi Family Farms, Salinas CA, U.S.A.), was purchased two days prior to testing from a retail grocery chain located in the Kansas City metropolitan area, and stored at 0.5 °C and 95% RH until the day of the study.

Testing Procedures

Descriptive Sensory analysis

A panel of six highly trained descriptive panelists from the Center for Sensory Analysis and Consumer Behavior (located in Manhattan, KS) participated in this study. The panelists had completed more than 120 hours of descriptive training, averaging more than 2,000 hours of testing experience (as documented by their participation in previous descriptive analysis projects in the Center for Sensory Analysis and Consumer Behavior (e.g. Chanadang et al., 2018, 2016; Swaney-Stueve et al., 2019), and had prior experience testing vegetables and vegetable products (Talavera-Bianchi et al., 2010b). The panel evaluated color, flavor and texture characteristics of the three spinach categories. Before evaluation started, the panel met for three hours divided across two sessions to review the existing lexicon previously developed to describe the flavor of a variety of fresh leafy vegetables (Talavera-Bianchi et al., 2010b). For this study, the panel reviewed the existing terminology, adjusted the terminology to focus only on fresh spinach and added a texture term (Initial Crispness) (Table 4.1). All the other attributes included were from the previous lexicon developed by Talavera-Bianchi et al. (2010). Panel performance was assessed using PanelCheck Software Version 1.4.0 (Nofima, Norway) and panel effects and panel*product interactions were observed for some of the examined attributes. Specifically there was a panel effect on overall green, lettuce, earthy, toothetch, and astringent and panel*product interactions for spinach, woody, water-like, overall sweet, sour, and bitter. This occurrence was expected due to the variable nature of the samples evaluated (Moskowitz et al., 2008; Zhang et al., 2018).

Table 4.1 Sensory modalities and attributes used in the descriptive sensory analysis of spinach.

Modality	Attribute	Definition	Reference
Texture	Initial Crispness	The intensity of audible noise at first bite with the molars.	Fresh Baby Spinach Leaf =2.5 Snow Pea = 8.0
Appearance	Green, Color	The intensity of green color.	PANTONE 2408 CP = 8.0 PANTONE 2410 C = 10.0
Flavor (including aromatics, mouthfeel, and basic tastes)	Green, Overall	Aromatic characteristics of plant-based materials. A measurement of the total green characteristics and the degree to which they fit together. Green attributes include one or more of the following: green-unripe, green-peapod, green-grassy/leafy, green-viney, and green-fruity. These may be accompanied by musty/earthy, pungent, astringent, bitter, sweet, sour, floral, beany, minty, and piney.	For reference see Talavera-Bianchi et al. (2010b)
	Green, Peapod	A green aromatic associated with green peapods and raw green beans; characterized by increased musty/earthy.	
	Green, Grassy/Leafy	A green Aromatic associated with newly cut-grass and leafy plants; characterized by sweet and pungent character.	
	Green, Viney	A green aromatic associated with green vegetables and newly cut vines and stems; characterized by increased bitter and musty/earthy character.	
	Lettuce	Green, slightly musty and sometimes bitter water-like aromatics associated with lettuce like Bibb and Iceberg.	

	Spinach	The brown, green, slightly musty, earthy aromatics associated with fresh spinach.	
	Parsley	The clean fresh green, bitter, pungent aromatics associated with fresh parsley.	
	Woody	Brown, musty aromatics associated with very fibrous plants and bark.	
	Musty/Earthy	Aromatics associated with damp, wet soil	
	Water-like (mouthfeel)	Liquid perception during mastication of some fruits and vegetables such as watermelon, peaches, tomatoes, and lettuce.	
	Tooth-etch (mouthfeel)	A chemical feeling factor perceived as drying/dragging when the tongue is rubbed over the back of the tooth surface.	
	Sweet, Overall	Aromatics associated with the impression of sweet substances such as fruit or flowers.	
	Sour	The fundamental taste sensation of which citric acid is typical.	
	Bitter	A basic taste factor of which caffeine is typical.	
	Salty	The fundamental taste factor of which sodium chloride in water is typical.	
	Umami	Flat, salty flavor enhances naturally occurring in some tomatoes.	
	Astringent	The drying, puckering sensation on the tongue and other mouth surfaces.	

For the descriptive testing, the samples were served at room temperature, plain with no additional dressings or flavors added. Each sample served to panelists consisted of 2-3 plain fresh leaves depending on size (about 10 grams per serving), placed on a 6-inch paper plate and identified with a 3-digit code. Panelists evaluated each sample monadically in three replications following a randomized design. Attribute intensities for each of the samples were evaluated using a 15-point scale where 0 means “none” and 15 means “extreme”.

Consumer testing

The consumer study was conducted in April 2017, at the Kansas State University Olathe Campus (Olathe, KS). The survey was completed voluntarily by 205 participants (mixture of males and females ranging in age from 8 years old to 80 years old) during a University social event. Participants were screened only by their willingness to taste fresh spinach with a light dressing. Participants blind tasted the three types of spinach samples monadically following a randomized complete block design and rated their acceptance on the basis of appearance, overall liking, flavor liking and texture liking using a 9-point hedonic scale ("Dislike Extremely" to "Like Extremely"). Participants were also asked to rate their perception on the color intensity, overall flavor, thickness and texture of the spinach leaves using a 5-point Just-About-Right (JAR) Scale as described by Cadot et al. (2010). Lastly, they were asked to rank the three samples in order of preference. Participants were provided with bottled drinking water and saltine crackers for cleansing their palate between samples.

For the consumer testing, each sample was served at room temperature with a light vinaigrette dressing, which consisted of three parts of extra virgin olive oil, one part white wine vinegar, salt and pepper (6.5 g salt and 1.35 g pepper for every 200ml of vinaigrette). This dressing type was used for simulating the experience of eating a spinach salad without compromising substantially the organoleptic characteristics of the samples. The spinach leaves and dressing were mixed within 5 minutes of serving, in a ratio of 29.5 ml of dressing per 100 grams of spinach. Each sample served to consumers consisted of 2-3 leaves (depending on leaf size) placed on a 6-inch paper plate. Each participant received a tray containing one plate of each sample organized in the sample testing order (based on a previously determined design) with a plastic fork and knife. Participants proceeded to evaluate each sample left to right following a sequential monadic method. Participants used iPads to complete the questionnaire for each sample. They were instructed which sample to evaluate and when to cleanse their palate through the questionnaire. Data was collected electronically using Compusense Cloud (Compusense, Inc, Guelph, Ontario).

Statistical analysis

Data from consumer testing were analyzed using XLSTAT Version 19.02 (XLSTAT Data Analysis and Statistical Solution for Microsoft Excel. Addinsoft, Paris, France). Analysis of variance (ANOVA) was used for mean separation of liking scores. Penalty Analysis on overall liking scores was conducted for JAR questions as described by Rothman et al. (2009). The mean drop was calculated by subtracting the mean liking of the JAR group from the mean liking of each non-JAR group. Thereafter, the mean drop was plotted with the percentage of subjects giving each response. Penalties with a mean drop higher than 1.5 and with a response rate higher than 20% were taken into consideration. Friedman's Test was conducted for ranking data. Data from the descriptive sensory analysis were analyzed using SAS (Version 9.2, Cary, N.C., U.S.A.). Analysis of variance (ANOVA) with Fisher's LSD method was used for descriptive data mean separation.

Results and Discussion

Descriptive Sensory analysis

A total of 19 sensory attributes were examined. The panelists determined that 18 attributes could be used to describe color, flavor and texture characteristics in all three spinach samples, while one attribute (lettuce) was found only in the spinach grown in high tunnel and in the commercially-grown spinach (Table 4.2). The Parsley attribute has been used previously for describing spinach samples (Talavera-Bianchi et al., 2010b) but was not found in any of the samples in this study and for that reason not included in Table 4.2. Eight attributes were determined to be significantly different ($p \leq 0.5$). The panel did not detect any differences between samples for the flavor attributes green overall, green peapod, lettuce, woody, musty/earthy, tooth etch, sour umami and astringent, as well as initial crispness. Spinach grown in the high tunnel demonstrated high intensity in green, grassy/leafy, green, viney, water-like, sweet, overall, bitter and salty, which indicates the high organoleptic quality of this sample, (The only exception was green color, where open-field spinach was rated significantly higher). The commercially-grown spinach demonstrated significantly lower values for the green color, green/grassy/leafy, green, viney and spinach attributes when compared to the locally-grown spinach from the high tunnel and open-field. The spinach grown in open-field was rated significantly lower in the water-like, sweet overall and salty attributes when compared to the spinach grown in high tunnel and commercially-grown spinach. Open-field spinach was significantly more bitter than the commercially-grown spinach, and slightly (although not significantly) more bitter than spinach grown in the high tunnel.

Table 4.2 Descriptive analysis attribute mean[1] scores and standard deviations of spinach grown in high tunnel, open-field and commercially-grown spinach.

Modality	**Attribute**	**Sample**			**p-value**
		High Tunnel	**Open-field**	**Commercially-grown**	
Appearance	Green Color	9.0 b (N/A)[2]	10.0 a (N/A)	7.8 c (N/A)	< 0.0001
Texture	Initial crispness	2.7 (0.41)	2.9 (0.65)	2.6 (0.49)	0.170
Flavor	Green, Overall	4.9 (0.68)	4.9 (0.52)	4.7 (0.53)	0.080
	Green, Peapod	2.2 (0.45)	2.2 (0.38)	1.9 (0.23)	0.051
	Green, Grassy/ Leafy	4.2 a (0.42)	4.3 a (0.45)	3.8 b (0.51)	0.002
	Green, Viney	2.1 a (0.40)	2.1 a (0.36)	1.5 b (0.6)	0.001
	Lettuce	0.1 (0.34)	0.0 (0.0)	0.1 (0.34)	0.58
	Spinach	3.6 a (0.47)	3.6 a (0.44)	3.1 b (0.28)	0.000

	Woody	1.3 (1.08)	1.4 (0.93)	1.3 (0.84)	0.965
	Water-like	2.8 a (0.42)	2.3 b (0.44)	2.9 a (0.46)	< 0.0001
	Musty/ Earthy	2.3 (0.56)	2.4 (0.40)	2.4 (0.37)	0.333
	Tooth-etch	2.6 (0.80)	2.6 (1.04)	2.5 (0.75)	0.921
	Sweet, Overall	1.0 a (0.84)	0.4 b (0.64)	0.9 a (0.88)	0.005
	Sour	1.1 (0.74)	1.3 (0.77)	1.2 (0.61)	0.627
	Bitter	2.8 ab (0.63)	3.0 a (0.56)	2.4 b (0.50)	0.010
	Salty	1.1 a (0.62)	0.6 b (0.72)	1.2 a (0.50)	0.003
	Umami	2.4 (0.27)	2.5 (0.35)	2.5 (0.42)	0.661
	Astringent	2.4 (0.52)	2.3 (0.45)	2.2 (0.53)	0.292

1. means with different letters within a column are significantly different ($P \leq 0.05$) according to Fisher's protected least significant difference (LSD) test
2. The evaluation for color was done by consensus so there is no standard deviation

The low intensity of the organoleptic attributes of the commercially-grown-spinach could be attributed to the handling of the product through the distribution chain. The principal factor affecting produce quality is temperature (Lee and Kader, 2000). In big commercial operations, due to the large volumes of produce that are handled, delays in pre-cooling and packaging often exceed 12 hours (Kim et al., 2005), exposing the product in non-optimum temperatures. This exposure continues throughout transportation, where temperature discrepancies from the optimum storage temperature can reach up to 13°C (Koseki and Isobe, 2005) and in retail display where the discrepancies can reach up to 20°C (Nunes et al., 2009). Generally, every 10°C increase in temperature accelerates the rate of deterioration by 2- to 3-fold and loss in nutritional quality of fresh produce (Kader, 1988). Spinach packaged in micro-perforated bags and stored at 20°C demonstrated faster chlorophyll, carotenoid and folate degradation when compared to spinach stored at 4 or 10°C (Pandrangi and LaBorde, 2004). During storage, nutrient deterioration due to non-optimum temperatures may also negatively affect the flavor quality of fresh produce (Kader, 2008). In this study, the locally-grown spinach was precooled immediately and stored at optimum temperature until the test day. In fact, access to cooling facilities is a major challenge for scaling-up local food production in the region (Greater Kansas City Food Hub Working Group, 2015).

Moreover, the time between harvest and consumption is negatively correlated with flavor retention (Kader, 2008). Unfortunately, this time period is unknown for the commercially-grown spinach. The local spinach was harvested three days before the test and the commercially-grown spinach was purchased two days prior to the test. Typically, commercially produced fruits and vegetables may spend up to 5 days in transit and 1 to 3 days on retail display (Barrett, 2007). Nevertheless, this study provides a "real life" scenario in which the production to consumption distance is significantly shorter in local food systems when compared to non-local food systems (La Trobe and Acott, 2000). Local farmers are able to plan their harvest close to the time of retail sales (e.g. farmers market or community-supported agriculture (CSA) sales), while non-local produce must travel from the point of production to the point of consumption. Some of these factors could explain why the commercially-grown spinach demonstrated significantly less intensity in green/spinach flavors. Factors like cultivar, fertilization, microclimate, and postharvest climate can have an effect on organoleptic quality (Zhao et al., 2007b). In the case of the commercially-grown spinach, the specifications of the growing system/methods and spinach cultivar used for commercially-grown spinach sample are unknown. The locally-grown spinach was cultivated using standard growing practices for the region (Buller et al., 2016).

Consumer Testing

The consumer acceptance scores indicate that the spinach grown in high tunnels was liked significantly more in terms of overall liking ($p<0.0001$), flavor ($p<0.0001$), and texture ($p=0.004$) when compared to the spinach grown in open-field and the commercially-grown spinach (Table 4.3). Participants mean overall liking scores, flavor liking scores, and texture liking scores of the spinach grown in open-field and the commercially grown spinach were similar (Table 4.3). The three samples had similar appearance liking scores (Table 4.3). When forced to rank the samples based on preference, there was no significant difference across the three samples at 95% confidence, but there was a directional preference for spinach grown in high tunnel (p=0.069). Earthy et al. (1997) demonstrated that positioning overall preference questions after attribute rating questions has an effect on preference, which could explain the discrepancy between overall liking and preference ranking in this study.

Table 4.3. Average acceptance scores and standard deviations for appearance, overall liking, flavor and texture. For each attribute, means associated with different letters are significantly different.

	Overall Liking	**Appearance**	**Flavor**	**Texture**
High Tunnel Spinach	6.5 a (1.9)	6.2 a (1.8)	6.4 a (2.0)	6.3 a (1.9)
Open-field Spinach	5.8 b (2.1)	6.5 a (1.9)	5.7 b (2.2)	5.8 b (1.9)
Commercially-grown Spinach	5.7 b (2.1)	6.3 a (1.9)	5.5 b (2.2)	5.8 b (1.9)
P-value	< 0.0001	0.269	< 0.0001	0.004

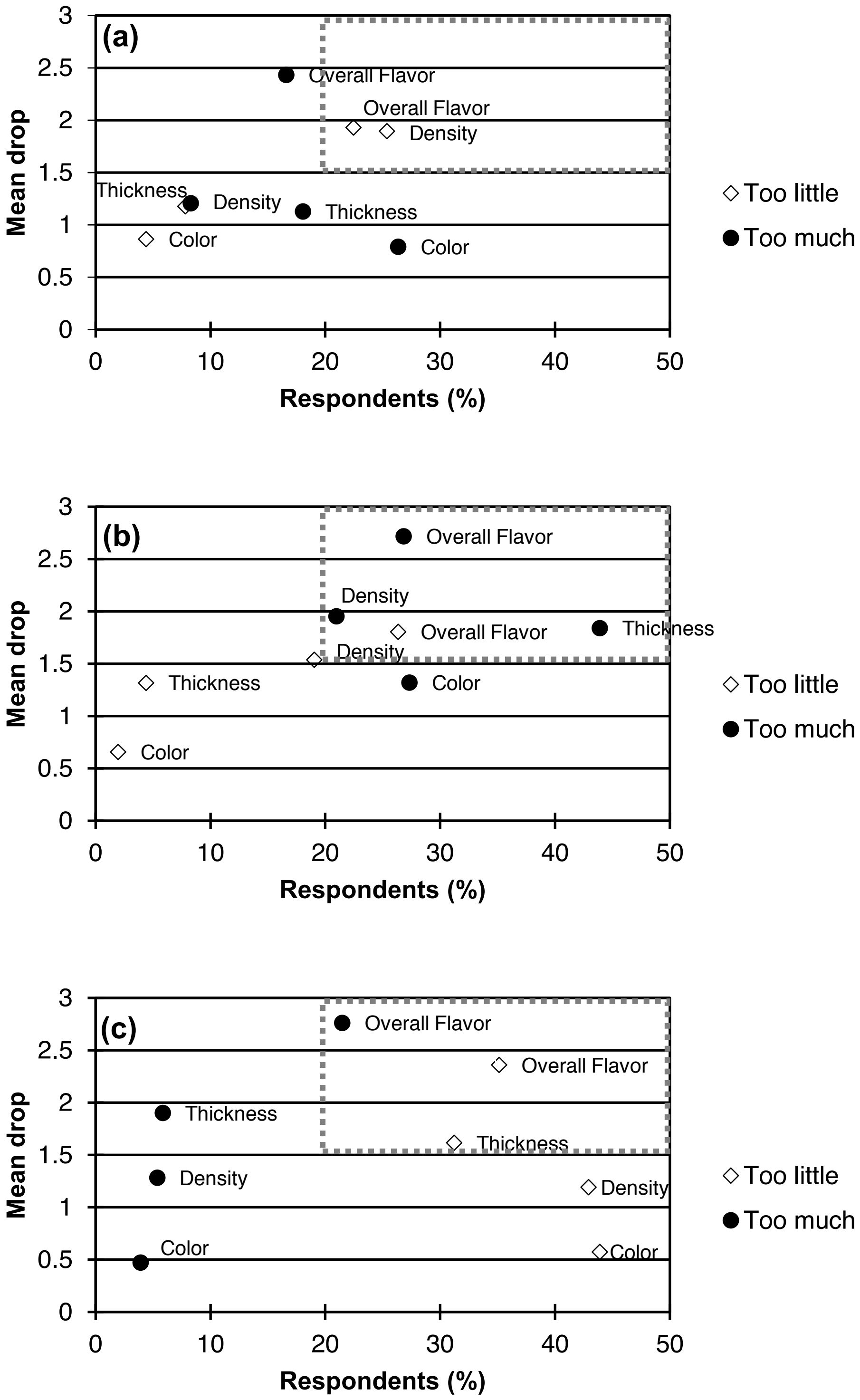
Using the Just-about-Right data, Penalty analysis/mean drop analysis was conducted to examine the relation between the JAR rating score of the attributes with the overall liking. (Rothman et al., 2009). It shows to what extent overall liking was penalized by the not-JAR respondents (Cadot et al., 2010). Spinach grown in high tunnel had the least number of penalties, which is consistent with the higher rating of overall liking received. Based on penalty analysis for the high tunnel grown spinach, the lack of overall flavor and low crispiness/crunchiness had the biggest (negative) effect on liking (largest mean drop) (Figure 4.1a). Despite the penalty for low crispiness/crunchiness, the consumers liked the most in terms of texture the spinach produced in the high tunnel (Table 4.3). This finding might be linked with the increased tenderness of the spinach produced in the high tunnel, caused by higher leaf water content (Batziakas et al., 2019a).

Penalty analysis for the open-field sample showed that this sample had significant penalties for too much overall flavor, lack in overall flavor, being too crunchy/crisp and too thick (Figure 4.1b). While spinach grown in open-field received penalties for being too weak and too strong in overall flavor, it had a much higher penalty for having too strong overall flavor (Figure 4.1b). This is in accordance with the sensory analysis results which showed that this sample was the least sweet and water-like, had directionally higher bitterness and significantly more green color. Open-field production has been found to produce spinach with higher antioxidant capacity when compared to high tunnel (Zhao et al., 2007c) and antioxidant compounds can contribute to a bitter taste (Drewnowski and Gomez-Carneros, 2000). Moreover, increased color intensity has been related to increased flavor intensity (Spence, 2015), thus the greener color of open-field spinach might have contributed to the penalties for strong overall flavor. (Talavera-Bianchi et al., 2010a) demonstrated that there is a negative correlation between green color, water-like and bitter taste versus sweet taste which is in accordance with the findings for the open-field spinach and commercially-grown spinach. Sweetness and bitterness in vegetables can predict positive and negative preference respectively (Dinehart et al., 2006). Bitterness is a characteristic that can strongly influence liking (Drewnowski, 1997; Dinehart et al., 2006) and when present in vegetables it is linked with bad taste and dislike (Drewnowski, 1997). Sweetness (Smith et al., 1982) and saltiness (Sharafi et al., 2013) can suppress/mask bitterness to some extent (Smith et al., 1982; Sharafi et al., 2013). This may suggest why high tunnel spinach was liked more in terms of flavor and overall liking compared to open-field spinach; they were not significantly different in bitterness but the high tunnel spinach was significantly sweeter and saltier and demonstrated less green color compared to open-field spinach. The penalties that open-field and high tunnel spinach received for crunchiness/crispiness are contradictive to the descriptive analysis findings for this attribute, which showed no significant difference in initial crispness between the three samples. (Barcenas et al., 2004) argues that trained panels decrease individual differences and is suggesting, for eliminating bias and inaccurate conclusions, using naïve consumer panels coupled with trained sensory panels.

Commercially grown spinach received low JAR ratings for all the evaluated attributes (data not shown) and the descriptive analysis showed that this sample demonstrated significantly less intensity in green/spinach flavors. This could explain why the commercially grown spinach was not liked as much as the high tunnel sample, despite the fact it was as sweet and not bitter. Penalty analysis showed that this sample received high mean drops for having too thin leaves (Figure 4.1c) and for overall flavor, both too strong and not strong enough (Figure 4.1c). The variation in leaf thickness between the samples might be a result of plant leaf adaptation to the different growing conditions (Witkowski and Lamont, 1991). Specifically for spinach, fertilization, cultivar and leaf maturity have an effect on leaf thickness (Gutiérrez-Rodríguez et al., 2013).

It appears that in this study there are three consumer flavor preference segments. One segment that perceived the spinach flavor as too weak. Another found it “Just About Right” and one that perceived flavor as too strong. A similar segmentation was found for arugula (Fouladkhah et al., 2011) and spinach acceptance is influenced by individual taste perception (Turnbull and Matisoo-Smith, 2002). Specifically, consumers with genetic sensitivity to bitter taste compounds have a lower taste detection threshold (Drewnowski, 1997), which can be reflected to increased dislike for spinach consumption (Turnbull and Matisoo-Smith, 2002). This could also explain why all the spinach samples received penalties for being both too strong and not strong enough in flavor.

Figure 4.1 Penalty analysis of spinach grown in high tunnel (a), open-field (b), and commercially-grown spinach (c). Penalties with a mean drop higher than 1.5 and with a response rate higher than 20% are contained in the dashed line rectangle.


(a)
Overall Flavor
Overall Flavor
Density
Thickness
Density
Thickness
Color
Color
(b)
Overall Flavor
Density
Overall Flavor
Thickness
Density
Thickness
Color
Color
(c)
Overall Flavor
Overall Flavor
Thickness
Thickness
Density
Density
Color
Color
Mean drop
0
0.5
1
1.5
2
2.5
3
Respondents (%)
0
10
20
30
40
50
◇ Too little
● Too much

The spinach samples in this study were served with a light vinaigrette dressing to avoiding masking the spinach flavor with the dressing flavor and same time simulate a realistic consumer scenario (e.g. spinach consumed raw in salad with dressing). Nevertheless, it is unknown if all the participants were familiar with this type of dressing, or if they are typically consuming spinach with a stronger tasting type of dressing (e.g. ranch dressing) which can dominate the flavor palate. It has been found that serving raw broccoli with ranch dressing to children, increased their liking and consumption of this vegetable (Fisher et al., 2012). Liking of a seasoning/flavoring is related to familiarity with the seasoning (Pliner and Stallberg-White, 2000) and absence of the familiar flavoring can evoke a poor reaction to taste (Rozin and Rozin, 1981). The flavor segmentation might be also related to participants' different levels of familiarity with spinach. Sensory preference of food is directly related to consumption experience (Bingham et al., 2005) and flavor preference is affected by various parameters including age, health status and food neophobia (Tuorila, 1996). The recruiting criteria for this study was broad; willingness to taste spinach. Thus, this study might have included a broad spectrum of participants with various consumption frequency patterns. Moreover, it is unknown if the participants were frequent consumers of locally or non-locally produced spinach with specific expectations for fresh spinach. (Bingham et al., 2005) found that the liking score for spinach is directly related to consumption frequency. The results of this study show that locally-produced spinach demonstrated better organoleptic characteristics and that local high tunnel produced spinach was preferred by the consumers in terms of flavor when compared to the commercially-grown spinach. However, the attribute of flavor is of varying importance in different market niches. Consumers that primarily seek convenience are typically not considering the flavor of minimally processed vegetables as important (e.g. ready to eat leafy greens)

(Ragaert et al., 2004), which suggests that the flavor attribute will not greatly affect their purchasing decision.

Conclusions

This study presented a descriptive sensory analysis and a consumer acceptance test of spinach grown locally using two different growing systems, high tunnels and open-field, in addition to non-local, commercially-grown spinach. Spinach produced locally in high tunnels was liked the most amongst the consumers scoring significantly higher in overall liking, flavor and texture and received the least penalties amongst the three samples. The consumer liking scores of spinach produced locally in open-field and non-local commercially-grown spinach were similar. The descriptive sensory analysis indicates that locally grown spinach demonstrated high intensity in a set of attributes that suggest a product with high organoleptic quality. On the contrary, commercially-grown, store bought spinach demonstrated lower intensity in these attributes. The results of this study indicate that producers/retailers of locally grown spinach may develop marketing strategies that are aiming specifically to the consumer niche that is seeking products of high organoleptic quality. A future study should consider using commercially-grown non local spinach of the same variety, utilizing same growing system/method and that is harvested in the same day as the local spinach sample in order to determine a possible effect of cultivar, growing system/method and harvest day.

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Chapter 5 - Reducing postharvest losses of spinach stored at non-optimum temperatures with the implementation of passive modified atmosphere packaging

Abstract

Postharvest losses of fresh produce constitute the biggest portion of the total food losses occurring in food chains globally. The main driver behind the postharvest losses of fresh fruits and vegetables is temperature abuse occurring mainly during transportation and storage. This is a particular problem for small acreage producers, who frequently have limited access to postharvest handling resources like optimum refrigeration conditions. Passive MAP is a relatively inexpensive intervention that does not require specialized equipment and has demonstrated some potential for maintaining the quality and extending the shelf life of fresh produce stored in non–optimum temperatures. Our objective was to determine the effect of passive MAP on the quality and storage life of locally grown spinach (Spinacia oleracea cv. Corvair) when stored in non-optimum temperatures. Mature spinach leaves (≈320 g) were packaged in passive MAP bags, developed using the BreatheWay® technology, and non-MAP produce bags and subsequently stored at 13 °C or 21 °C. Spinach physical and nutritional quality was evaluated throughout its storage life in terms of overall visual quality, water loss, leaf tenderness, surface color, chlorophyll content, electrolyte leakage, chlorophyll fluorescence, antioxidant capacity, total phenolic content and vitamin C content. Spinach that was stored in MAP bags reached headspace equilibrium at approximately 6% O2 and 11% CO2 at 13 °C and approximately 4% O2 and 8% CO2 at 21°C after 2 days of storage for both temperatures. The spinach stored in passive MAP at 13 or 21 °C demonstrated significantly higher visual quality during storage and 2 and 1 day longer storage life, respectively, when compared to the control. The spinach in passive MAP demonstrated a slower rate of yellowing and water loss during storage. The limiting factor for the spinach stored in MAP was decay due to condensation at 13 °C and yellowing at 21 °C. There were no statistical differences in the examined nutritional quality parameters between the spinach stored in MAP and produce bags. This study shows that passive MAP can be a valuable tool for reducing the food losses occurring in small acreage fruit and vegetable operations that have limited access to cooling and refrigerated storage.

Introduction

Global food demand is constantly rising and is expected to increase by 110% in 2050 (Mc Carthy et al., 2018) due to the increase in global population and the improvement of the standard of living around the globe (Tilman et al., 2011; Mc Carthy et al., 2018; Porat et al., 2018). However, there are concerns about the ability of agricultural food systems to satisfy this increase in demand (Davis et al., 2016; Conijn et al., 2018). Reducing food losses and waste (FLW) is a major component of the efforts for achieving global food security (Shafiee-Jood and Cai, 2016; Xue et al., 2017; Porat et al., 2018). Approximately 30 to 50% of the food produced globally for human consumption is lost or wasted (Myers et al., 2017; Porat et al., 2018). Fresh produce constitutes the largest amount of FLW, reaching approximately 45% by weight (Lipinsky et al., 2013). Postharvest losses constitute a considerable portion of the FLW in fresh produce (Beretta et al., 2013; Porat et al., 2018) and they are related to the rate of biological deterioration of the commodity. Fresh fruits and vegetables naturally deteriorate after harvest, and the rate of deterioration is affected by a variety of factors including respiration, ethylene production and action, compositional changes, water loss, physiological disorders, and pathological breakdown (Kader, 2005, 2013). Produce that is subjected to non-optimum storage temperature and humidity, mechanical damage and improper packaging demonstrates increased rate of deterioration, which can lead to rapid quality loss (Kader, 2005; Prusky, 2011). The latter can lead to product discarding or reduced consumer acceptability (Shewfelt et al., 2014) and consumer dissatisfaction is one of the main drivers of postharvest food waste in fresh produce (Baldwin, 2002; Kader, 2005).

Modified atmosphere packaging (MAP) is a postharvest intervention that has been successfully implemented for reducing postharvest losses by extending the shelf life and maintaining the quality of a variety of fruits and vegetables (Kader et al., 1989; Zhang et al., 2006; Cortellino et al., 2015; Mampholo et al., 2015; Domínguez et al., 2016; Luna et al., 2016). MAP mechanism involves reducing the O_2 and increasing the CO_2 levels in the atmosphere surrounding the product, which results in reducing its respiration rate and metabolic activity (Kader et al., 1989; Gorris and Peppelenbos, 1992). The two main types of MAP are active and passive modification of the atmosphere (Zagory and Kader, 1988). In active MAP, the desired atmosphere is established rapidly by flushing the desirable gas mixture in the package (Ghidelli and Pérez-Gago, 2018). In passive MAP, the desired atmosphere is established after a “lag” period, as a result of the synergistic effect of the package film permeability and the respiration rate of the commodity (Lange, 2000; Charles et al., 2008). Generally, active MAP can be regulated in a bigger extend, has an immediate impact on the packaged product (Kader and Watkins, 2000) and thus is more effective compared to passive MAP (Gil, 2016). However, it is an expensive technique that requires a high investment in equipment and gases (Rodriguez-Aguilera and Oliveira, 2009). Passive MAP does not require such investment, which constitutes it an intervention more suitable for smaller operations like small acreage farmers. Successful implementation of MAP is closely related to the package, the commodity and the storage environment. Factors affecting MAP include product respiration rate, ethylene production and sensitivity, storage temperature, and tolerance to low O_2 and high CO_2 levels (Zagory and Kader, 1988). Particularly for passive MAP, a crucial factor affecting its efficacy is the oxygen transmission rate (OTR) of the packing film due to its central role in atmosphere establishment (Lange, 2000).

Spinach, *Spinacia oleracea* is a cold weather crop, that is rich in micronutrients like folate and carotenoids and also has high antioxidant capacity (Pandrangi and LaBorde, 2004; Pandjaitan et al., 2005; Bunea et al., 2008). In 2016, United States was the second largest spinach producer in the world (FAO, 2019). Spinach production in the U.S. has been steadily increasing, mainly due to a rise in consumer demand driven by increased consumer health awareness (Morelock and Correll, 2008). It is a highly perishable crop (Kader, 2002a) and its optimum storage conditions are 0°C and 95-98 % RH (Suslow and Cantwell, 1999). Spinach has been reported to benefit from a MAP with 1-3% O_2 and 8-10% CO_2 when stored 0 °C (Suslow and Cantwell, 1999). Spinach stored in (non-MAP) perforated bags at 16 or 20 °C had a shelf life of 4 days for both temperatures, while at 12 °C the shelf life was 6 days, and at 1 °C the shelf life was more than 16 days (Kou et al., 2014).

Spinach is a crop that is particularly popular among small acreage growers in the Central US, particularly in the Central U.S. (Knewtson et al., 2010). One of the main challenges that small acreage growers are facing in this region is access to cooling (Greater Kansas City Food Hub Working Group, 2015; Chiebao et al., 2018). MAP could be a solution for these growers, but it can only have a supplemental effect to temperature management (Kader et al., 1989). Adequate temperature management is the most important and effective tool for shelf life extension and quality maintenance of fresh produce (Lee and Kader, 2000; Saltveit, 2003; Prusky, 2011; Kader, 2013). Moreover, MAP is typically designed for optimum storage temperature and when used at other temperatures it can have adverse effects to the packaged product (Brecht et al., 2003). An increase in storage temperature may result in a damaging atmosphere for the packaged product due to O_2 depletion and CO_2 accumulation, because the product respiration increases but the packaging film gas permeability does not respond equivalently (Beaudry et al., 1992; Exama et al., 1993). The O_2 and CO_2 tolerance limits vary between commodities (Kader et al., 1989; Gorny, 2003; Mangaraj et al., 2009; Sandhya, 2010), but out of tolerance exposure of a commodity, will commonly result in off-odor and off-flavor development due to anaerobiosis along with discoloration (Kader et al., 1989; Beaudry, 2000; Watkins, 2000; Shayanfar, 2013). Furthermore, the increase in temperature itself accelerates the deterioration rate of the packaged produce (Kader, 2002), which in combination with the unfavorable atmosphere can rapidly diminish product quality.

While MAP is not a substitute for proper temperature management (Lange, 2000), it may have potential to benefit stored spinach when the cooling capabilities are limited and could be a prospective solution for reducing postharvest spinach losses for growers who lack proper cooling and storage facilities. A beneficial effect of MAP for produce that is stored in non-optimum temperatures has been reported for a variety of crops (Fonseca et al., 2005; Løkke et al., 2012; D’Aquino et al., 2016; Murmu and Mishra, 2017) including spinach (Medina et al., 2012; Garrido et al., 2016; Mudau et al., 2018). The main challenge for applying passive MAP in non-optimum storage temperatures is finding a film with appropriate OTR that can match the respiration rate of the stored product (Lange, 2000) and create a beneficial atmosphere for the product. BreatheWay® membrane technology is an innovative approach for creating a beneficial passive MAP in non-optimum temperatures (Lange, 2000; Clarke, 2011; Wilson et al., 2019).

This membrane utilizes side chain crystalline polymers (SCC) spread on a microporous substrate (Clarke, 2011). This membrane has OTR and carbon dioxide transmission rate (CTR) approximately 1000 times greater than standard 2-mil polypropylene (PP) film (Clarke, 2011), and can further adjust its permeability when exposed to increased temperature (Lange, 2000; Wilson et al., 2019). This specific property constitutes mechanism by which the BreatheWay® membrane is able to be suitable for passive MAP designed for both optimum and non-optimum temperatures. The BreatheWay® technology has been shown to be successful in maintaining the quality of broccoli (Clarke, 2011) and ackee fruit (Emanuel et al., 2018), thus it could be a solution for creating a beneficial passive MAP for spinach stored in non-optimum temperatures.

The overall objective of this work was to study the potential benefits of passive modified atmosphere packaging, using the BreatheWay® technology, on the postharvest losses of spinach when stored at non – optimum temperatures. More specifically, we evaluated the effect of passive MAP when spinach was stored at non- optimum temperature on its storage life, and its physical, organoleptic and nutritional quality.

Materials and Methods

Plant Material

Spinach (*Spinacia oleracea* cv. Corvair) was grown in open field during spring of 2018, at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC), located in Olathe, KS (lat 38.884347 N, long 94.993426 W). Mature and defect-free spinach leaves were harvested and immediately transferred, using insulated coolers, to the postharvest physiology lab at the K-State Olathe campus. In the lab, the spinach was washed three times in ice-cold tap water and sorted a second time to remove defective leaves. Next, the spinach was centrifuged using a 5-gallon salad spinner (Chef Master 90005, China) for removing excess water before packaging.

Packaging and Storage Conditions

The modified atmosphere (MA) was established passively using a microporous membrane (OTR ~5×10^6 cc m^{-2} day^{-1}) with semipermeable polymer coating (BreatheWay®, Curation Foods, Santa Maria, CA) attached over a 12.7-mm diameter hole in a PP/PE (polypropylene/Polyethylene) laminate bag (30 cm length × 30 cm width). The membrane was temperature specific and was developed exclusively for this spinach variety for storage at 13 °C and 21 °C, using respiration measurements of this variety from previous trials. These temperatures where selected to reflect the storage temperatures used by local growers in the Central U.S. Small acreage growers use 13 °C for comingling chilling sensitive and non-sensitive crops and when there is no access to refrigeration they use A/C cooled rooms at 21 °C (personal communication). The target atmosphere was 3-6 % O_2 and 6-12% CO_2 for both temperatures. Approximately 320 grams of spinach were weighed and placed into the MAP bags. Consequently, the bags were heat-sealed using a tabletop impulse heat-sealer. For the control treatment, approximately 320 grams of spinach were weighed and placed in plastic produce bags. The produce bags were not sealed but they were folded to limit water loss, which is a common practice amongst local growers. The MAP and control bags were stored in environmental chambers (ThermoFisher Scientific Inc., Asheville, NC, USA), in two different temperature conditions; at 13°C with 95% RH and 21°C with 95% RH. The analyses were performed destructively on days 0, 3, 5, 7 and 9 for 13°C and on days 0, 1, 2, 3, 4 and 5 for 21°C. For both treatments, there were 3 replicates per day of analysis, prepared on day 0 and then randomly assigned for each analysis day. The experiment was conducted twice using spinach harvested from the same plot.

Spinach Quality Analyses

Package Headspace Composition

The headspace composition in the MAP and produce bags was determined by measuring O_2, CO_2 and ethylene concentrations. A portable gas analyzer (Bridge Analyzer; Bedford Heights, OH, USA) was used to measure the O_2 and CO_2 concentration. Ethylene was measured by extracting a sample of 1 ml from the headspace of each bag using a syringe. The sample was injected onto a gas chromatograph (SRI Instruments, Torrance, CA,USA) fitted with an FID with 10 ppb limit of detection and equipped with a 6' HAYESEP-D stainless steel column (100/120 mesh). The injector, column and detector temperatures were set at 125 °C, respectively and helium was used as the carrier gas at a flow rate of 20 ml min^{-1}.

Physical Quality

The physical quality of the spinach during storage life was evaluated by measuring overall visual quality (OVQ), water loss, leaf surface color, leaf tenderness, electrolyte leakage, chlorophyll fluorescence, and chlorophyll content.

Spinach OVQ was measured similarly to Medina et al. (2012). For each treatment, 20 leaves per replicate were evaluated visually by two trained analysts, taking into account freshness, appearance, color, and uniformity and rated from 9 = "excellent" to 1 = "extremely poor;" the limit of product marketability was considered 5 = "Fair". When 30% of the treatment scored below 5, the treatment was terminated, indicating the end of storage life.

The water loss of the stored spinach leaves was evaluated similarly to Agüero et al. (2008). The spinach packaged in each treatment was weighed on day 0 (W_0) of each experiment and on each analysis day (W_A). The water loss of each treatment was calculated using the formula: Water Loss (WL %) = $(1 - W_0/W_A) \times 100$.

The tenderness of the spinach leaves was measured using a texture analyzer TA-58, TA.XT.plus (Texture Technologies Corp., Scarsdale, NY, USA), equipped with a 5-blade TA-91 Kramer Shear Cell (Texture Technologies). The return distance of the probe was set at 35 mm, the test speed at 1.7 mm/s, and the return speed was set at 10 mm/s. For each measurement, the midribs of 10 leaves per replication were removed and the leaves were stacked and weighed. The maximum force required to cut through the spinach leaf stack was measured similarly to Prakash et al. (2000) and Gomes et al. (2008). Leaf tenderness was calculated as the maximum force in Newtons per gram (N/g).

The color of five leaves per replicate for each treatment was measured using an A5 Chroma-Meter Minolta CR-400 (Minolta Co. Ltd., Osaka, Japan). The color measurements were taken on the upper part of the leaf at two opposite points to the central leaf axis. Color results are expressed in the CIE L*a*b* color space were L* is lightness and h° is hue angle, calculated as $\tan^{-1} b^*/a^*$ (McGuire, 1992).

The extraction and quantification of the spinach leaf chlorophyll content was performed according to the method developed by Wellburn (1994). On the day of the physical quality analysis, samples of 0.3 g of spinach leaf fresh tissue from each replicate were rapidly frozen using liquid nitrogen and stored at -20˚C in the dark until the extraction day. Chlorophyll was extracted by homogenizing the plant tissue in 10 ml of pure methanol using a benchtop homogenizer (POLYTRON PT 1600 E, Kinematica AG, Luzern, Switzerland) and subsequently incubated at 4°C in darkness for 24 hours. After the incubation period, the samples were measured with a 96-well microplate reader spectrophotometer (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA) at 653 nm (Chlorophyll b) and 666 nm (Chlorophyll a). The total chlorophyll content was calculated using the following equations: Total chlorophyll content = Chl a + Chl b and Chlorophyll a (Chl a): [15.65 x (A666) - (7.34 x (A653)]; Chlorophyll b (Chl b): [27.05 x (A653) - (11.21 x (A666)]. The total chlorophyll content was expressed as mg/100 g FW.

The electrolyte leakage of the stored spinach was measured by modifying the method described by Bajji et al. (2002). For each replicate, 20 leaf disks obtained from 10 leaves (diameter 10 mm) were placed in 20 ml of deionized water in a 50-ml tube and the electrolyte leakage was measured immediately (this value is denoted as EC_0) using a conductivity meter (OHAUS Corporation, Parsippany, NJ, USA). Thereafter the tubes were incubated at 21 ˚C on a platform shaker (Thermo Fisher Scientific, Waltham, MA, USA) at 30 rpm for 2 h and after that period the measurement was repeated (this value denoted as EC_1). Subsequently, the samples in tubes were stored at -20 ˚C until the final measurement day. Electrolyte leakage measured after thawing (this value is detnoted as EC_{max}) is assumed to be 100% for the given sample. Prior to the measurement of ECmax, the tubes were incubated on a platform shaker at 80 rpm until the samples reached 21 ˚C. The electrolyte leakage was expressed as a percentage, calculated using the following formula: Electrolyte leakage (EL %) = $[(EC_1 - EC_0)/EC_{max} - EC_0] \times 100$.

Chlorophyll fluorescence was determined using a pulse modulated OS30p+ chlorophyll fluorometer (Opti-Sciences Inc., Hudson, USA). Three leaves per replicate were used for this measurement. Dark adaptation was accomplished, as proposed by Maxwell and Johnson (2000), using FL-DC clips (Opti-Sciences Inc., Hudson, USA) attached on the leaves for 30 minutes prior the measurement. Minimal fluorescence (Fo) and maximal fluorescence (Fm) were analyzed and the ratio Fv/Fm of variable fluorescence (Fv) to maximal fluorescence (Fm) was calculated by the instrument.

Nutritional Quality

The nutritional quality of the stored spinach was evaluated by measuring total phenolic content, antioxidant capacity, and vitamin C content. On the days of the physical quality analyses, spinach leaves were immediately frozen using liquid nitrogen and thereafter stored at -20°C for the nutritional quality analyses. The frozen spinach samples were freeze-dried (Harvest Right Freeze Drier, North Salt Lake, UT, USA) and then pulverized using a pestle and mortar. Each time the water content was calculated by weighing each sample before and after freeze drying. The extraction method used for determining antioxidant capacity and total phenolic content was described by Huang et al.,(2002); 0.2 grams of spinach powder were homogenized in centrifuge tubes with 20 ml of acetone/water (1/1) solution. The mixture was shaken at 80 rpm for 1 h using a platform shaker (Thermo Fisher Scientific, Waltham, MA, USA) and then centrifuged (JA-17, Beckman Coulter, Palo Alto, CA, USA) for 20 minutes at 4°C and 17,600 x g_n. The supernatant was used for the antioxidant capacity and total phenolic content analyses.

The antioxidant capacity of the samples was determined by measuring the Ferric Reducing Ability of Plasma (FRAP) and the Oxygen Radical Absorbance Capacity (ORAC). The FRAP measurement was performed following the method described by Benzie and Strain (1996). Absorbance at 593 nm was measured against a trolox positive control using a spectrophotometer equipped with a 96-well microplate reader (Synergy H1, BioTek Instruments, Inc. Winooski, VT, USA). FRAP was expressed as μM trolox equivalent in 100 g fresh weight basis (μM TE/100 g FW). The ORAC measurement was performed according to the method described by Cao et al.

(1993) and modified by Ou et al. (2001) and Prior et al. (2003) using a spectrophotometer equipped with a 96-well microplate reader (Synergy H1). The antioxidant activity was correlated with the oxidative damage to the fluorescent probe against a trolox positive control. ORAC was expressed as µM trolox equivalent on a 100 g fresh weight basis (µM TE/100 g FW).

The total phenolic content of the spinach leaves was determined using the method developed by Singleton and Rossi (1965). Using a spectrophotometer equipped with a 96-well microplate reader (Synergy H1), the extracted samples were measured at 750 nm absorbance. Total phenolic content was expressed as mg gallic acid equivalents on a 100 g fresh weight basis (mg GAE/100g FW).

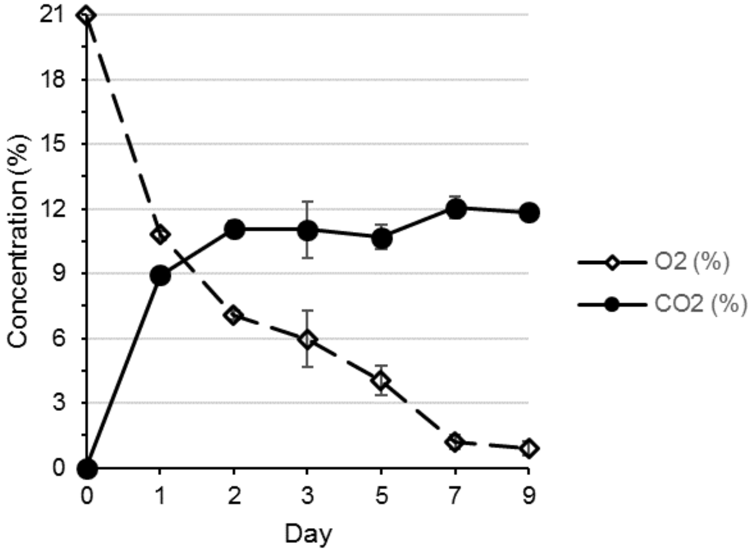
On the days of the physical quality analyses, following the method of Klimczak and Gliszczynska-wiglo (2015), 2 g of fresh spinach leaves from each replication were homogenized with 20 ml of an acid solution (6% metaphosphoric acid/ 2N glacial acetic acid) using a homogenizer (POLYTRON). Subsequently, the samples were stored at -20˚C and analyzed later using ultra performance liquid chromatography (UPLC). On the days of analysis, after thawing, the samples were centrifuged at 8500 x g_n for 10 min (JA-17, Beckman). The supernatant was further diluted with the acid solution and then filtered with a 0.2 µm NYL w/GMF Whatman syringe filter (Whatman Inc, Clifton, NJ, USA). An Acquity PDA QDa Waters UPLC (Waters Corp., Milford, MA, USA) equipped with an Acquity BEH C18 column (Waters Corp., Milford, MA, USA) was used for the analysis. The injection volume was 5 µL and the flow rate was set at 0.2 mL/min. The mobile phase was composed of 5 mM potassium phosphate monobasic (KH_2PO_4), pH 2.65 with 0.1% of formic acid (solution A) and methanol with 0.1% of formic acid (solution B). The linear gradient of the mobile phase was programmed as follows: 5-15% B in 1 min, followed by 15-35% B for 1 min and return to initial conditions in 4 min. A photodiode array detector (PDA) was used for measuring vitamin C at 245nm. The quantification of vitamin C was performed using a five-point standard curve (2.5 µg/mL - 50 µg/mL) with purified ascorbic acid (assay percentage range ≥99.0 %, Fisher Scientific, Hampton, NH, USA) as a standard. The vitamin C content was expressed as mg ascorbic acid on a 100 g fresh weight basis (mg AA/100 g FW). In the cases that the vitamin C content was below the quantification limit (0.024 µg/mL), the half value of the quantification limit was used in the subsequent statistical analysis.

Results

Quality of spinach during storage at 13 °C

The spinach stored in passive MAP reached a headspace equilibrium atmosphere of approximately 6% O_2 plus 11% CO_2 after 2 days of storage at 13 °C (Figure 5.1). The O_2 concentration in the MAP started declining again from day 5 on, reaching approximately 0.5% on day 7 and remained at this level until day 9, while the CO_2 concentration increased to 12% on day 7 and remained steady until the end of the storage life (Figure 5.1). The headspace in the produce bag was measured at the same time intervals with the MAP headspace and it was found to be the same as ambient air (≈ 20.95% O_2 and 0.04% CO_2). There was no ethylene detected in either the MAP or produce (control) bags during storage.

Figure 5.1 Headspace composition of spinach packaged in passive modified atmosphere packaging (MAP) and stored at 13 °C for 9 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.



Overall quality-The spinach stored in MAP at 13 °C maintained significantly higher overall visual quality compared to the spinach stored in produce bags from day 5 of storage until the end of shelf life on day 9 (Figure 5.2). The spinach stored in the produce bags was unmarketable after 7 days at 13°C while the spinach stored in MAP was unmarketable after 9 days (Figure 5.2). There was no difference in water loss between the two treatments for the first 7 days of storage, but by day 9 the spinach in MAP had lost 1.5% less weight ($P < 0.01$) than the spinach in produce bags (Figure 5.2). There was no difference in leaf tenderness between the two treatments (Table 5.1). The leaf color did not differ between the two treatments until after 5 days of storage at 13°C (Figure 5.3). However, the spinach in MAP was darker than the spinach in the produce bags on days 7 and 9 ($P < 0.05$ and $P < 0.001$, respectively) (Figure 5.3). On day 9, the spinach in MAP maintained higher hue values compared to the spinach in the produce bags ($P < 0.05$) (Figure 5.3). The chlorophyll content of the spinach in MAP was 28% higher than the spinach in the produce bags ($P < 0.001$) (Figure 5.4). The spinach packed in MAP had 5% and 6% less electrolyte leakage on days 7 ($P < 0.05$) and day 9 ($P < 0.01$), respectively, compared to spinach stored in the produce bags (Figure 5.4). The MAP packaged spinach also had lower Fv/Fm values ($P<0.05$) compared to spinach in produce bags on days 5 and 7; however, there was no difference in chlorophyll fluorescence on the last day of the storage life (Figure 5.5). When examining the nutritional quality parameters, ORAC, FRAP, total phenolic content, and vitamin C, there were no significant differences between the two treatments (Table 5.1).

Figure 5.2 Overall visual quality (OVQ) and water loss of spinach packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 13 °C for 9 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.

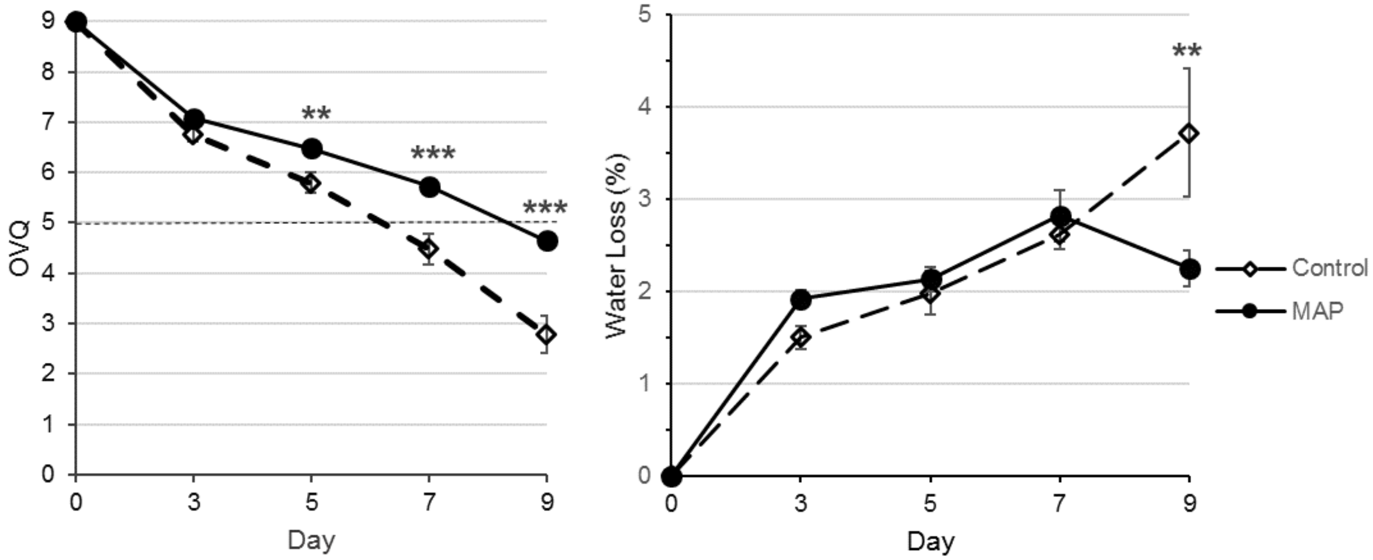


Table 5.1 Probability values reflecting the effects of storage day, treatment, and their interactions on overall visual quality (OVQ), water loss, leaf tenderness, leaf color, chlorophyll content, electrolyte leakage, chlorophyll fluorescence, total phenolic content, antioxidant capacity, and vitamin C content of spinach stored in passive modified atmosphere packaging (MAP) or produce bags at 13 °C for 9 days.

	P-value

Parameter^{zy}	Storage Day	Treatment	Storage Day× Treatment
OVQ	<.0001	<.0001	<.0001
Water loss	<.001	NS^{x}	<.05
Leaf tenderness	<.0001	NS	NS
Color-lightness	<.0001	<.001	<.05
Color-hue	<.0001	<.05	<.05
Chlorophyll content	NS	NS	NS
Electrolyte leakage	<.05	<.05	NS
Chlorophyll fluorescence	<.0001	<.01	NS
ORAC	NS	NS	NS
FRAP	<.0001	NS	NS
Total phenolic content	NS	NS	NS
Vitamin C	<.0001	NS	NS

[z] A linear mixed model was used to test which factors and interactions between factors had significant effects on the examined quality parameters (α=0.05).

[y] The analysis included two trials performed with the same experimental conditions.

[x] NS = Non-significant

Figure 5.3 Lightness and hue angle of spinach leaves packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 13 °C for 9 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.

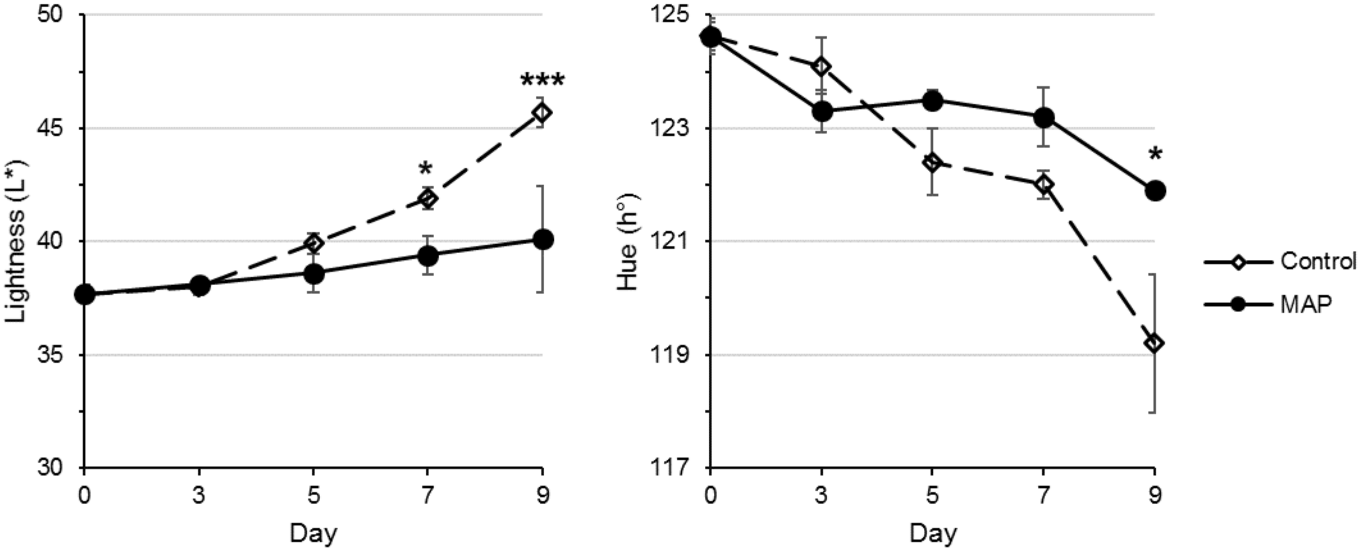


Figure 5.4 Chlorophyll content and electrolyte leakage of spinach leaves packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 13 °C for 9 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.

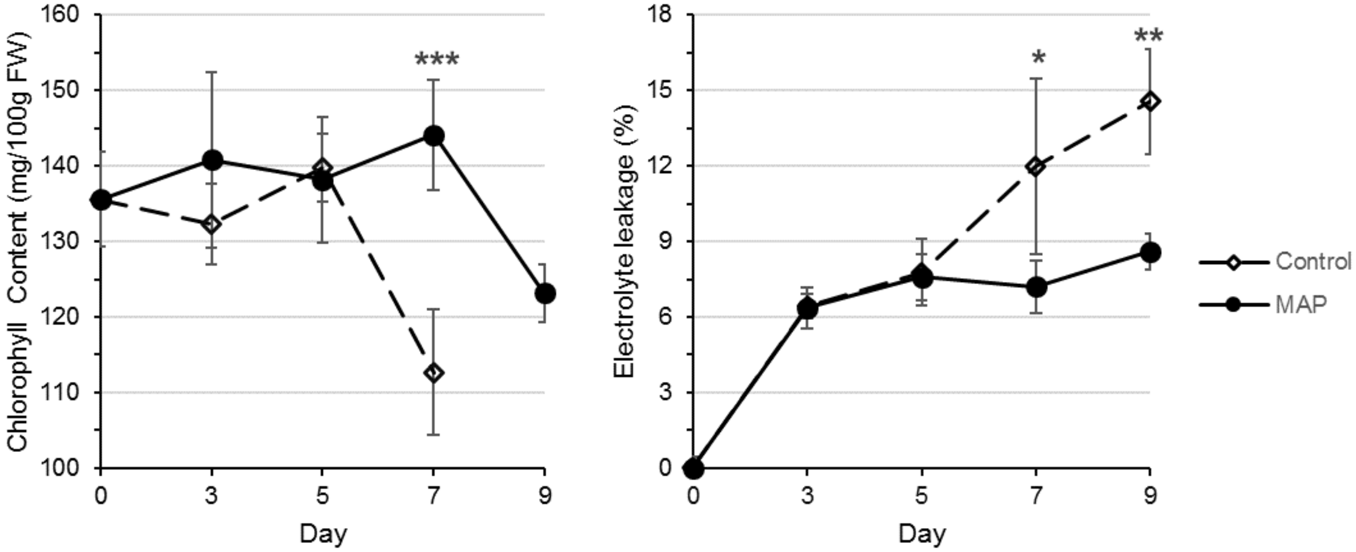
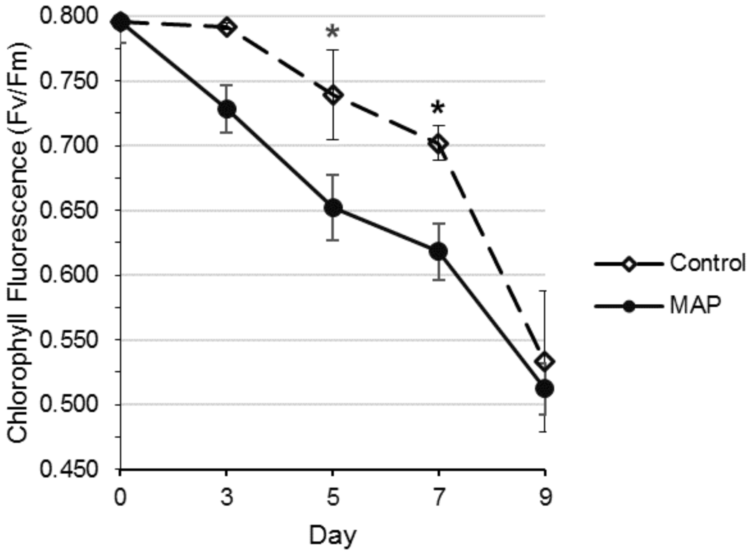


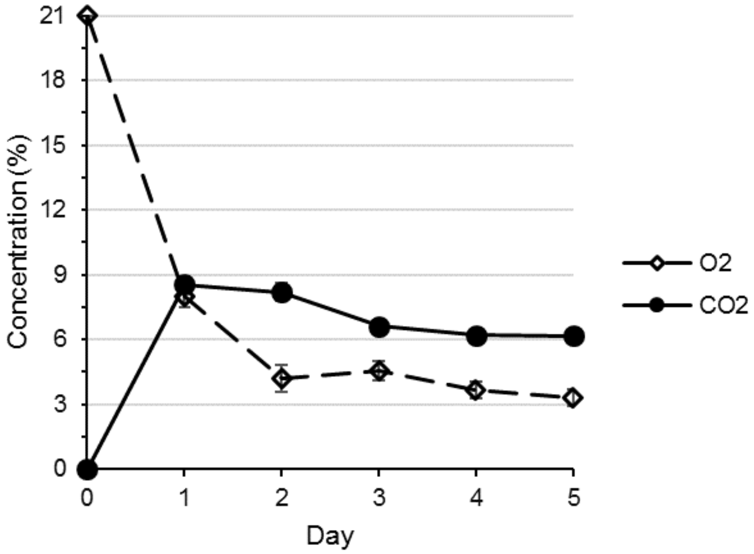
Figure 5.5 Chlorophyll fluorescence of spinach leaves packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 13 °C for 9 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.



Quality of spinach during storage at 21 °C

The spinach stored in the passive MAP reached an equilibrium atmosphere of approximately 4% O_2 plus 8% CO_2 after 2 days of storage at 21 °C (Figure 5.6). The CO_2 concentration in the MAP started declining again from day 3 on, reaching approximately 6% on day 5 while the O_2 declined slightly to 3% (Figure 5.6). The headspace in the produce was measured at the same time intervals with the MAP headspace and it was found to be same as the ambient air (data not shown). There was no ethylene detected in either the MAP or produce bags during the storage life.

Figure 5.6 Headspace composition of spinach packaged in passive modified atmosphere packaging (MAP) and stored at 21 °C for 5 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.



The spinach stored in MAP maintained significantly higher overall visual quality compared with the spinach stored in produce bags from day 2 of storage until the end of storage life on day 5 (Figure 5.7). The spinach stored in the produce bags was unmarketable after 4 days of storage at 21 °C. There was no difference on water loss between the two treatments for the first 3 days of storage but the spinach in MAP had lost 1.4% (P <0.01) and 1% (P <0.05) less water than the spinach in produce bags on days 4 and 5, respectively (Figure 5.7). There was no difference in leaf tenderness between the two treatments (Table 5.2). The leaf color did not differ between the two treatments after 3 days of storage at 21°C (Figure 5.8). On days 4 and 5, the spinach in MAP had darker leaves compared with the spinach in produce bags (P <0.05) (Figure 5.8). On day 5 of storage the spinach in MAP had higher hue values, indicating more green versus yellow color compared to the spinach in produce bags (P<0.05) (Figure 5.8). However, there was no difference in the chlorophyll content between the two treatments (Table 5.2). The spinach packed in MAP demonstrated 1.7% higher electrolyte leakage on day 3 (P<0.05) compared with spinach stored in produce bags, but for the rest of the storage life there was no difference in electrolyte leakage between the two treatments (Figure 5.9). The MAP-packaged spinach also had higher

Fv/Fm values (*P*<0.001) than the spinach in produce bags on day 5 (Figure 5.9). There were no differences in the nutritional quality between the two treatments as defined by ORAC, FRAP, total phenolic content, and vitamin C (Table 5.2).

Figure 5.7 Overall visual quality (OVQ) and water loss of spinach packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 21 °C for 5 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.

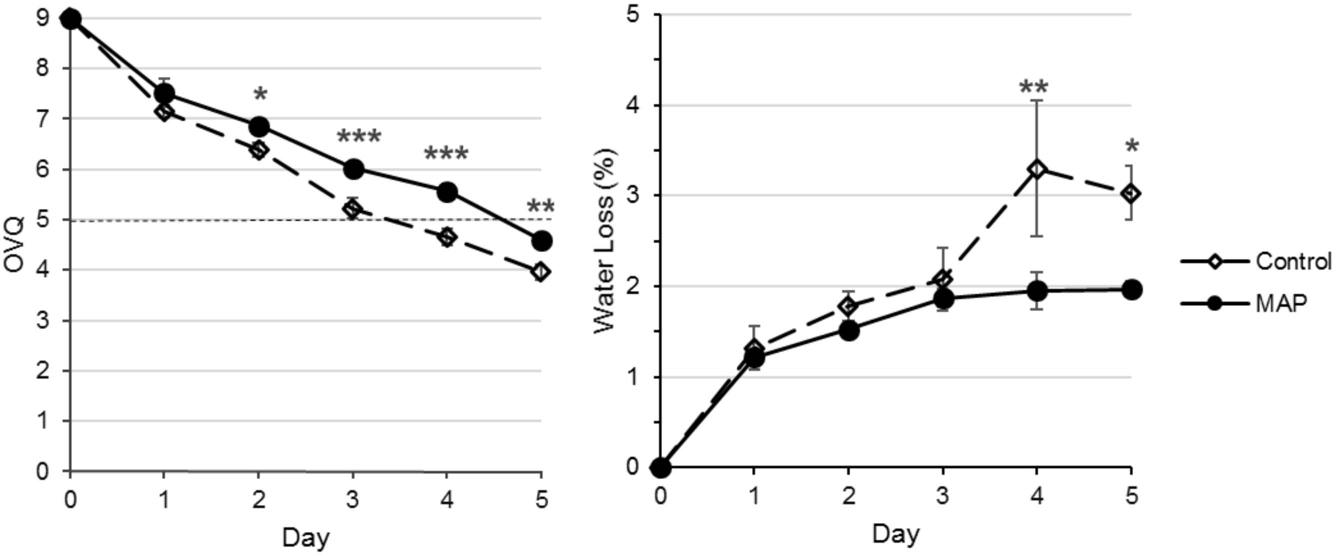


Table 5.2 Probability values reflecting the effects of storage day, treatment, and their interactions on overall visual quality (OVQ), water loss, leaf tenderness, leaf color, chlorophyll content, electrolyte leakage, chlorophyll fluorescence, total phenolic content, antioxidant capacity, and vitamin C content of spinach stored in passive modified atmosphere packaging (MAP) or produce bags at 21 °C for 5 days.

	P-value		
Parameter^{zy}	Storage Day	Treatment	Storage Day× Treatment

OVQ	<0.0001	<0.0001	NS
Water loss	<0.001	<0.001	NS
Leaf tenderness	<0.01	NS[x]	NS
Color-lightness	<0.0001	<0.01	NS
Color-hue	<0.0001	NS	NS
Chlorophyll content	NS	NS	NS
Electrolyte leakage	<0.01	<0.05	NS
Chlorophyll fluorescence	<0.0001	NS	<0.05
ORAC	<0.0001	NS	NS
FRAP	NS	NS	NS
Total phenolic content	NS	NS	NS
Vitamin C	<0.0001	NS	NS

[z] A linear mixed model was used to test which factors and interactions between factors had significant effects on the examined quality parameters (α=0.05).

[y] The analysis included two trials performed with the same experimental conditions.

[x] NS = Non-significant

Figure 5.8 Lightness and hue angle of spinach leaves packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 21 °C for 5 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.

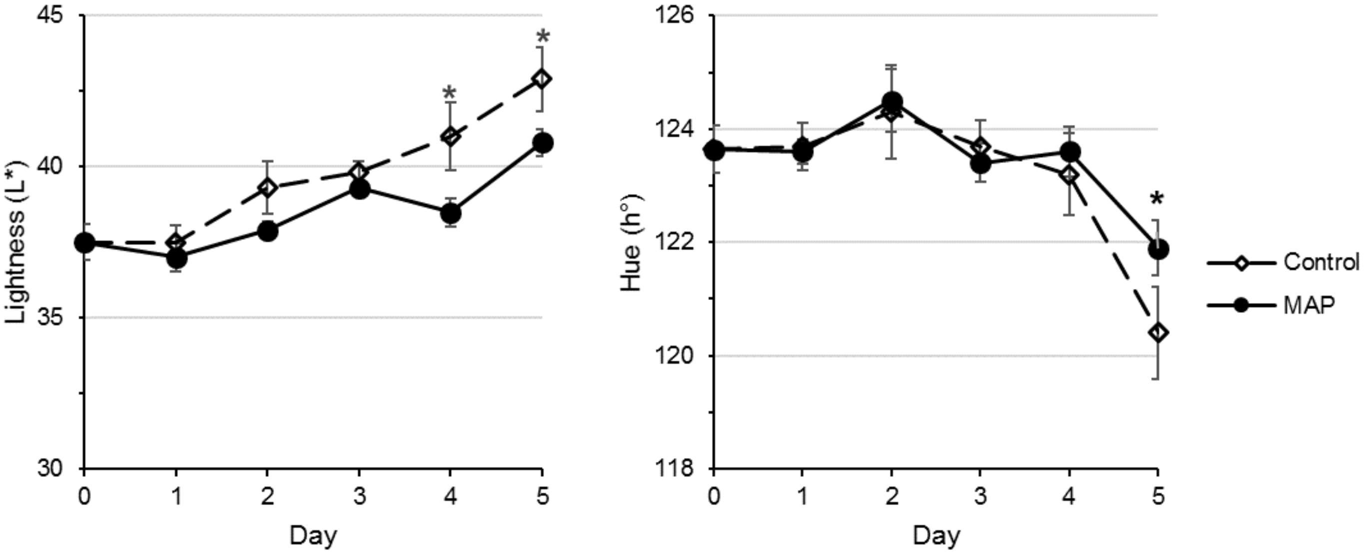
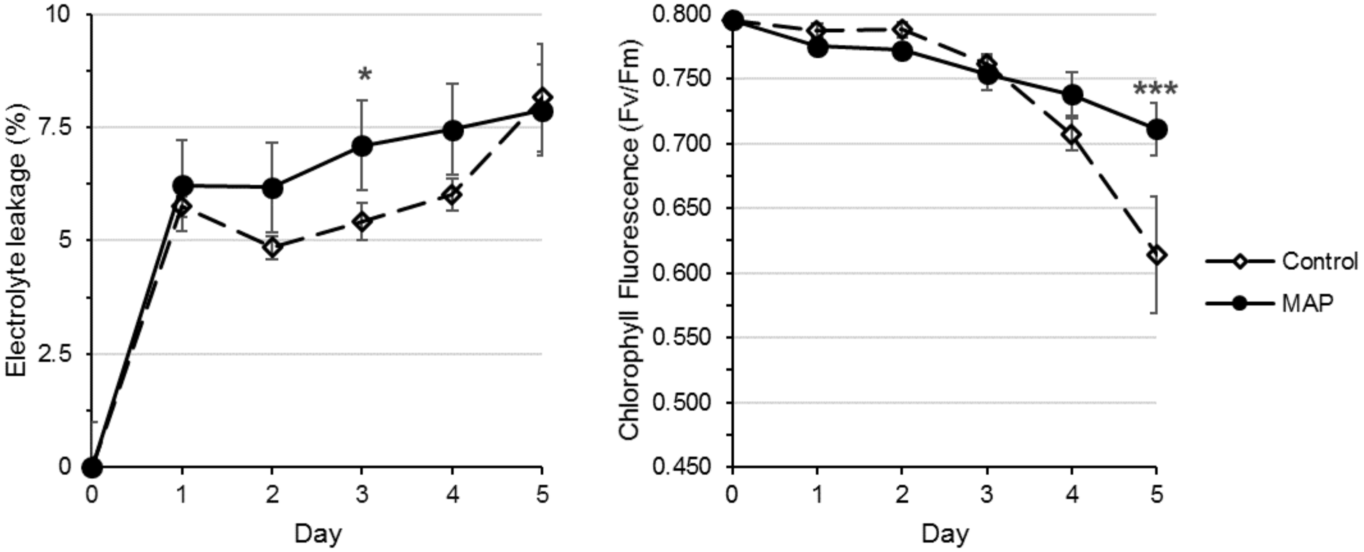


Figure 5.9 Electrolyte leakage and chlorophyll fluorescence of spinach leaves packaged in produce bags (Control) or passive modified atmosphere packaging (MAP) and stored at 21 °C for 5 days. Each value represents the mean (±Std Error) of measurements obtained from two trials with the same experimental conditions with 3 replicates in each trial.



Discussion

The goal of this work was to study the effect of passive MAP on the postharvest losses of spinach when stored at non-optimum temperatures as evaluated by storage life, physical quality, and nutritional quality.

The benefits of MAP applied at optimum storage temperatures have been well documented (Kader et al., 1989; Sandhya, 2010; Ghidelli and Pérez-Gago, 2018; Wilson et al., 2019). Temperature control remains the most effective postharvest management technique for reducing the rate of deterioration of fresh fruits and vegetables (Brecht, 1995; Paull, 1999; Lee and Kader, 2000; Saltveit, 2003; Prusky, 2011; Kader, 2013). However, MAP can only provide complementary benefits to temperature control (Kader et al., 1989; Sandhya, 2010). All the metabolic processes and physiological responses in plant tissues are strongly affected by their surrounding temperature (Kader and Saltveit, 2003; Nunes and Emond, 2002). Such processes and responses include respiration, ethylene production, and sensitivity to ethylene (Kitinoja and Kader, 2003; Prusky, 2011), water loss, compositional changes, and physiological breakdown (Paull, 1999, Prusky, 2011). However, maintaining the optimum storage temperature is not always feasible, especially in smaller horticultural operations (Cantor and Strochlic, 2009; Harrison et al., 2013). The optimum postharvest temperature for leafy greens is near 0 °C, but there have been MAP designs that have shown benefits at non-optimum storage temperatures (Fonseca, 2006; Løkke et al., 2012; Mudau et al., 2018). The shelf life of shredded kale (*Brassica oleracea var. acephala DC.*) was extended for 2 days when stored in MAP versus air at 20 °C to simulate retail handling conditions (Fonseca et al., 2005). Wild rocket (Diplotaxis tenuifolia L.) maintained good visual quality after 4 days of MAP storage at 10 °C (Løkke et al., 2012), while the shelf life of spinach stored in MAP at 20 °C was extended for 3 days (Mudau et al., 2018). Our results indicate that passive MAP created using the BreatheWay® technology is beneficial to the postharvest quality and storage life of spinach at non-optimum storage temperatures. The spinach store in our passive MAP design maintained higher overall visual quality beginning on day 2 of storage at 21 °C and day 3 at 13 °C until the end of the storage period. Consequently, the storage life of spinach stored in passive MAP was extended for 1 day at 21 °C and 2 days at 13 °C.

One of the main challenges in designing a MAP bag for storage at non-optimum temperatures is coupling the film permeability with the respiration rate of the product in order to create a non-injurious but also beneficial (to the product) atmosphere (Exama et al., 1993). As reported by Løkke et al. (2012) the majority of the MAP configurations used for storage of wild rocket at 10 or 20 °C developed injurious O_2 and CO_2 concentrations, which led to anaerobiosis and off odor development, or they created a non-injurious but not beneficial headspace. Similarly, Rux et al. (2017), reported injurious headspace conditions for rucola (i.e., rocket) stored at 20 °C. Mudau et al. (2018) reported CO_2 levels up to 18% for spinach stored in MAP at 20 °C. Spinach packed in passive MAP created using an EVOH/polyolephin film and stored at 13 °C or 28 °C had <1% O_2 and >30% CO_2 after 3 and 1 days, respectively (Park et al., 2006) and packaging spinach in MAP with such headspace led to off-odor development (Tudela et al., 2013). Spinach stored at 7°C in passive modified atmosphere (steady state at 0.5% O_2 and 10% CO_2) was of acceptable visual quality after 10 days but developed off-odors (Garrido et al., 2016). In this specific study, the atmosphere was modified using a polypropylene film (PP) that had low OTR compared to other available films (Yaptenco et al., 2007) and, as a result, the atmosphere reached the lower O_2 injury threshold for spinach (Beaudry, 2000). Medina et al. (2012) reported similar results to Garrido et al. (2016), with spinach stored at 7°C in passive MAP using a PP film resulting in off-odor development after 12 days. In our experiments, the atmosphere created using the BreatheWay® technology had an equilibrium of 4% O_2 plus 8% CO_2 at 21°C and 6% O_2 plus 11% CO_2 at 13 °C. These atmospheres are within the non-injurious O_2 (Beaudry, 2000) and CO_2 (Cantwell et al., 2010) limits reported for spinach. However, at 13 °C in this research, the O_2 concentration reached <1% after 7 days of storage accompanied by off-odor development. Off-odor development is a major problem in MAP spinach storage and it is linked to low O_2 levels in the packaging (Tudela et al., 2013).

We believe that the O_2 reduction during the latter part of the storage period was related to the appearance of decay in the MAP bags, which was the limiting factor at 13 °C, and was caused by water condensation. The majority of the polymeric films used in MAP, including PP and PE films, have a low water vapor transmission rate (WVTR) (Tano et al., 2007, Bhatia et al., 2013). Consequently, storing produce in MAP at non-optimum temperatures can create high humidity conditions in the package and condensation development (Brecht et al., 2003; Tano et al., 2007; Sandhya, 2010), which may result in decay development (Ben-Yehoshua et al., 1998). This was the case in our experiment for the spinach that was stored at 13 °C. In order to reduce condensation and potentially improve the effectiveness of the MAP packaging, pairing the BreatheWay® membrane with a film with higher WVTR such as Nylon-6/PE film should be considered, since that film has the appropriate moisture transport properties for limiting water vapor build up (Lim et al., 1999).

The lower chlorophyll fluorescence values observed in MAP at 13 °C could probably be explained by the rapid reduction in O_2 concentration that we observed on days 5 and 7. Low Fv/Fm values are a good indicator of stress in leafy vegetables (Baldassarre et al., 2011), which means the spinach stored in MAP at 13 °C was likely undergoing physiological stress. However, the spinach stored in MAP at 13 °C demonstrated significantly lower electrolyte leakage and higher overall visual quality than the control treatment. In spinach, electrolyte leakage has been correlated with product quality (Kou et al., 2014). This disparity may be related to the fact that plant tissue stress is not always visible and its visual symptoms need a ‘lag’ storage phase to be demonstrated (Baldassarre et al., 2011). Schofield et al. (2005) reported a similar disparity: iceberg lettuce (*Lactuca sativa L.*) of similar visual quality at harvest, but different chlorophyll fluorescence, differentiated in visual and physical quality during storage in correlation with the chlorophyll fluorescence. The spinach stored in MAP at 21 °C in our experiments had higher chlorophyll fluorescence than the control treatment on the last day of its storage life, indicating a less stressed plant tissue, and there were no major differences in electrolyte leakage between the two treatments at this temperature.

The quality and shelf life of leafy green vegetables is predominantly determined by fresh appearance and crisp texture and subsequently by the rate of water loss and chlorophyll breakdown occurring in the commodity during storage (Cantwell and Kasmire, 2002). The main challenges in spinach storage at non-optimum temperatures are increased rates of water loss and yellowing (Koike et al., 2011). Spinach quality is particularly sensitive to water loss with approximately 3% water loss making this commodity unmarketable (Sams, 1999; Bartz and Brecht, 2002). In our experiments, the water loss of the spinach stored in passive MAP remained below 3% for the entire storage periods at 13 and 21 °C. Moreover, in both storage conditions, the spinach in MAP demonstrated significantly less water loss than the control treatment towards the end of storage. MAP reduces water vapor diffusion from plant tissues by maintaining a near saturated environment, leading to lower water loss (Serrano et al., 2006).

The textural properties of leafy green vegetables are directly related to water content and cell turgor (Sams, 1999). However, we did not detect differences in texture between the two treatments at either of the examined storage temperatures. Medina et al. (2012) also reported that spinach samples with differences in water content did not demonstrate differences in tenderness measured as maximum shear force (N).

The color of the commodity is one of the main factors affecting consumer purchase decisions for fresh fruits and vegetables, and yellowing of leafy green vegetables is the leading reason for loss of acceptability (Shewfelt, 2002). Maintaining the green color of spinach directly translates into maintenance of its commercial value (Kaur et al., 2011). Generally, the ability of modified atmosphere (MA), controlled atmosphere (CA) storage and MAP to slow down the rate of chlorophyll breakdown and yellowing has been reported for various green vegetables such as amaranth (*Amaranthus cruentus L.* Mampholo et al., 2015), broccoli (*Brassica oleracea L. var. Italica*; Serrano et al., 2006), pak-choy (*Brassica rapa var. chinensis*; O'Hare et al., 2000), and spinach (Suslow and Cantwell, 1999). Similarly, in our experiments the spinach stored in passive MAP maintained darker leaf color towards the end of the storage life at both temperatures compared with the spinach stored in the control bags, indicating a slower rate of yellowing. At 13 °C, the color measurements were in accordance with the chlorophyll content and the spinach stored in MAP maintained higher amounts of chlorophyll. At 21 °C, there were no differences in chlorophyll content between the two treatments. In some cases, the chlorophyll breakdown in spinach does not occur evenly over the leaf surface and yellowing occurs in a patchy pattern (Bergquist et al., 2006). This may lead to inconsistencies between colorimeter measurements and chlorophyll content, since the colorimeter measures only a portion of the spinach leaf while the chlorophyll content analysis involves sampling a larger portion of the plant tissue (Proulx et al., 2010).

Postharvest quality does not only involve physical characteristics that are visible to the consumer, but also includes the nutritional content, flavor (Kader, 2000), and safety (Bruhn, 2002) of the commodity. In this study, there were no significant differences in total phenolic content, antioxidant capacity, and vitamin C content between the two treatments at either of the examined temperatures. Gil et al. (1999) similarly reported that spinach stored in a passive MAP of 6% O_2 plus 14% CO_2 at 10 °C did not differ in ascorbic acid content from spinach stored in non-modified conditions. Mudau et al. (2018) reported that spinach stored in a MAP of 5% O_2, 15% CO_2 maintained higher antioxidant activity compared to air storage at 4 °C but not at 10 or 20 °C. Our study did not examine the effect of passive MAP on the flavor characteristics of the stored spinach. Future studies should include sensory analysis and consumer testing if possible, since repeat purchases of fresh produce depend on flavor (Kader, 2001; Baldwin, 2002; Mitcham, 2010).

One of the limitations of this study is that we did not evaluate the effect of the studied MAP designs on food safety. When designing passive MAP applications for non-optimum storage temperatures, the effect of the application on human pathogens should be considered. This is of particular importance for leafy greens since they have been closely associated with a multitude of severe disease outbreaks (Herman et al., 2015). Specifically, spinach was in the center of the deadly 2006 U.S. outbreak, where 100 hospitalizations and five deaths were caused by Shiga toxin-producing *Escherichia coli* (STEC) O157 infections (Wendel et al., 2009). Storing produce at non-optimum temperatures increases the risk for growth of human pathogens such as *Salmonella* and *E. coli* (Francis et al., 2012). Generally, MAP at non-optimum temperatures has failed to limit the growth of human pathogens on leafy greens, including spinach (Oliveira et al., 2015). While our results indicate that the utilization of passive MAP could be a potential solution for quality maintenance during non-optimum temperature storage of spinach, the food safety aspect of this intervention should be investigated.

Conclusions

The results of this study show that the implementation of passive MAP for non-optimum temperature storage has the potential to reduce postharvest losses of spinach by maintaining the quality and extending the storage life. The spinach stored in passive MAP at both 13 and 21 °C demonstrated significantly higher visual quality during storage and longer storage life when compared to the control spinach stored in non-MAP produce bags. The implementation of passive MAP resulted in a slower rate of yellowing and water loss for the packaged spinach. Our results indicate that the passive MAP prolonged the "fresh life" of spinach, as indicated by the higher OVQ, greener and more turgid leaves during storage compared to the control treatment. However, there were no statistical differences in the examined nutritional quality parameters between the two packaging treatments at either temperature. The effectiveness of the examined passive MAP design could be improved by using a film with higher WVTR since decay caused by condensation was a limiting factor. Temperature control remains the most effective postharvest intervention for maintaining the quality and extending the shelf life of fresh produce. However, our results show that passive MAP can be a valuable tool for reducing the food losses occurring in small acreage fruit and vegetable operations that have limited access to cooling. These growers tend to store their product in bulk (personal communication), thus future research should examine a passive MAP design for storing spinach in bulk. Further research is needed for evaluating the effect of this passive MAP design on the control and growth of human pathogens like *E. coli.* The efforts for reducing FLW and satisfying global food demand should utilize innovative approaches and passive MAP designed for non-optimum temperatures is one of them.

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Chapter 6 - Conclusions

This dissertation presented the results of four independent, but complementary studies that focused on reducing food losses and waste while improving the quality of locally-produced spinach. Three important parts of the food chain were examined; production, storage, and consumption. The first study investigated the effects of a high tunnel production system on the yield, preharvest losses, and quality at harvest of spinach when compared to production in the open field. The second reported the effects of the high tunnel production system on the storage quality and shelf life of spinach stored in optimum and non-optimum temperatures. The next chapter explored the effect of local open field and high tunnel production of spinach on the sensory characteristics and consumer acceptability when compared with commercially store bought spinach. Finally, the fourth study investigated the effect of passive modified atmosphere packaging on the quality and shelf life of spinach stored in non-optimum temperatures.

Our results indicate that the high tunnel production system can reduce the preharvest food losses of spinach as evaluated by crop productivity and marketability, as well as the physical quality of the spinach. The high tunnel production system increased the availability of spinach, which was demonstrated by the continuous production throughout the winter and subsequent increased overall yield. Moreover, the spinach grown in the high tunnel system had a higher proportion of marketable yield, which directly reflects a reduction of food losses of spinach. The spinach grown in high tunnels resulted in significantly higher (126% - 529%) marketable yield and higher percent marketability (11.5% - 26.5%) in three consecutive years (2014-2017) and higher (85%-443%) total yield in two (2015-2017) of the three years. In regards to quality at harvest, the spinach produced in the high tunnels had 30% to 50% larger leaves ($P < 0.001$) and 2.1% to 2.4% higher water content ($P < 0.001$) when compared to spinach grown in the open field. Spinach grown in the open field plots had significantly higher antioxidant capacity (ORAC & FRAP) in both of the examined years (2015-2017). There was an inconsistent effect on total phenolic content and ascorbic acid content, with these phytochemicals demonstrating significantly higher values for the open field plots in one of the two years examined. The spinach produced in both production systems was of superior quality as indicated by the physical and nutritional quality attributes measured on the day of harvest. Spinach grown in the high tunnels demonstrated characteristics, such as increased water content and reduced respiration, which indicate a potential for improved postharvest behavior.

We also demonstrated that high tunnel spinach production can reduce the postharvest food losses of this crop when stored in non-optimum temperatures and particularly at 13 °C. During both years, spinach grown in the high tunnels and stored at the optimum temperature (3 °C) had 1.4% to 2.4% significantly higher water content than spinach grown in open field plots. There was an inconsistent effect on total phenolic content, antioxidant capacity, and ascorbic acid content during storage at 3 °C. These nutritional quality parameters demonstrated significantly higher values for the open field plots during storage, but not in both years. However, there were no other major differences between the spinach produced in the two production systems during storage at the optimum temperature. The spinach produced in the high tunnels exhibited higher quality and longer shelf life when stored at 13 °C. During storage at 13 °C, in both years, spinach grown in the high tunnels had 1.2% to 2.3% higher water content than spinach grown in the open field. In the second year, high tunnel spinach stored at 13 °C had a lower respiration rate ($P < 0.05$) and a slower rate of yellowing as indicated by higher chlorophyll content ($P < 0.001$), significantly lower lightness values, and significantly higher hue values than open field spinach. As a result, the spinach grown in the high tunnels demonstrated longer shelf life in year 1 and higher quality towards the end of shelf life in year two when compared to open field spinach when stored at 13 °C. Similarly, to the storage experiment at 3 °C, there was an inconsistent effect on total phenolic content, antioxidant capacity, and ascorbic acid content during storage at 13 °C.

Additionally, the consumer and sensory study indicate that locally produced spinach, in high tunnels and open field plots demonstrated high intensity in a set of attributes that suggest a product with high organoleptic quality such as green color and green/spinach flavors. In contrast, non-local, commercially-grown spinach from California demonstrated lower intensity in these attributes as indicated by the descriptive sensory analysis. A consumer test showed that spinach grown in high tunnels had scored significantly higher in overall liking ($p<0.0001$), flavor liking ($p<0.0001$) and texture liking ($p<0.05$) when compared to open field locally produced and non-local store-purchased spinach. The consumer liking scores of spinach produced locally in open-field and non-local commercially-grown spinach were similar. This study indicates that local high tunnel production can decrease postharvest food waste of spinach by producing a crop of premium organoleptic quality that achieves high consumer acceptability.

Finally, the results of MAP study demonstrated that the implementation of passive MAP for non-optimum storage temperature has the potential to reduce the postharvest losses of spinach, by maintaining quality and extending storage life. Specifically, the spinach stored in passive MAP at 13 and 21 °C demonstrated significantly higher visual quality during storage and two and one days longer storage life, respectively, when compared to the control. The spinach stored in passive MAP demonstrated a slower rate of yellowing and water loss during storage. The limiting factor for the spinach stored in MAP was decay due to condensation at 13 °C and yellowing at 21 °C. There were no statistical differences in the examined nutritional quality parameters between the spinach stored in MAP and produce bags.

Overall, our results indicate that the high tunnel production system is suitable for local spinach production in the Central U.S.. We showed that production in high tunnels leads to a reduction in food losses and waste of spinach and that the product grown is of high organoleptic and nutritional quality. High tunnels will be an integral part of local food systems that focus on achieving sustainable production and food security. Our results further highlight the fact that temperature control is the most effective postharvest tool for maintaining the quality and extending the shelf life of fresh produce. However, innovative solutions such as passive modified atmosphere packaging can be a valuable tool for reducing food losses that occur in operations with limited access to cooling such as small acreage local production units. As the role of local production increases for addressing food security, it will be important to implement technologies that are able to produce high yields while maintaining good quality.