

EFFECTS OF TIME, CULTIVAR, AND STORAGE ENVIRONMENT
ON WINTER SQUASH IN SEMIARID MONTANA

by

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DEDICATION

First, I would like to thank God for instilling in me a love for His creation and wonder for the science and systems within it. He has provided all the things that have helped me get to where I am today.

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TABLE OF CONTENTS

1. WINTER SQUASH STORAGE AS AN OPTION FOR VEGETABLE FARMERS IN MONTANA	1
Introduction.....	1
Storage Environment and its Relationship to Fruit Quality:.....	6
Fruit Quality and Nutrient Composition:	7
2. CHAPTER TWO	13
Materials and Methods.....	13
Site Description.....	13
Crop Planting and Management.....	14
Squash Harvest.....	17
Storage Environments	17
Squash Varieties.....	18
Experimental Design.....	18
Chemical Composition Measurements	19
Statistical Analysis.....	20
Results and Discussion: The Effects of Cultivar, Time, and Storage.....	21
Results.....	21
Dry Matter.....	21
Starch Content.....	23
Soluble Solids Content.....	25
Mass	27
Color	30
Discussion	31
Cultivar	32
Time Period.....	33
Storage Environment	34
Conclusion	35
REFERENCES CITED.....	37

LIST OF TABLES

Table	Page
1. Table 1 Average Temperature and Precipitation by Month in Bozeman, MT.....	14
2. Table 2 Winter Squash grown and stored in our 2022-2023 trial by Species.....	15
3. Table 3 Winter squash production and harvest data.....	16
4. Table 4 Mean temperature and relative humidity of storage environments.....	18
5. Table 5 Means of DM content by variety, across both storage environments	21
6. Table 6 Means values of DM, Starch, and Soluble Solids by Variety	22
7. Table 7 Means of Starch content by variety, across both storage environments.....	24
8. Table 8 Means of SS content by variety, across both storage environments.....	26
9. Table 9 Total percent of mass lost by variety, in each storage environment.....	29

LIST OF FIGURES

Figure	Page
1. Figure 1 Dry Matter Content by Variety and Months of Storage	21
2. Figure 2 Starch Content by Variety and Months of Storage.....	23
3. Figure 3 Soluble Solids Content by Variety and Months of Storage.....	25
4. Figure 4 Change in Mass (g) by Variety and Months of Storage	28
5. Figure 5 Change in Mass (%) by Variety and Months of Storage.....	29
6. Figure 6 Change in a* Value by Variety and Months of Storage	31

ABSTRACT

Winter squash can be stored for months after harvest while taste, texture, and color attributes improve. The potential for producers to attain greater storage longevity and produce better squash would benefit farmers, consumers, and economies. Due to variation in local climates and the respective vendors' needs, understanding how to control a storage environment can be challenging. This research looks at how storage environment and storage period affect biochemical and physical attributes of ten varieties of winter squash in a semiarid climate. The varieties include three *Cucurbita pepo*, one *Cucurbita moschata*, one *Cucurbita maxima x moschata* hybrid, and five *Cucurbita maxima* cultivars. Two storage environments were used for analysis. One was located indoors and cooled to 53 degrees F while the other was built into an existing barn with a heating unit and insulation to keep the temperature above freezing. A total of 240 winter squash were placed in each storage space. A completely randomized design was used to assign categorical treatments of storage time to each winter squash, with six levels of treatment being implemented. Each variety of squash (n=4) was analyzed monthly in each storage environment for changes in soluble solids, dry matter, starch content, interior color and exterior color (using CIELAB color values L*, a*, b*), and mass. The temperature and relative humidity of each storage environment were also monitored and recorded hourly during the same period. Cultivar was always the most significant factor. There was no simple effect of the storage environment on dry matter, soluble solids, starch content, or mass. There was a strong significant interaction observed between time and cultivar for each response variable, which shows us that the type of squash and how long it should be stored for are important when discussing quality. Storage did have a significant effect on the a* value of interior color, which is an indicator of quality and nutritional value. The biochemical and physical attributes of each cultivar analyzed varied greatly in its response to the amount of time in storage which in turn influences the taste, texture, and sensory quality of each cultivar uniquely.

CHAPTER ONE

WINTER SQUASH STORAGE AS AN OPTION FOR VEGETABLE FARMERS IN

MONTANA

Introduction

Montana produces just under one percent of the United States agriculture goods (USDA ERS, 2023). Although the state is not known for its vegetable production, farmers are responsible for about \$47.5 million in vegetable sales (USDA NASS, 2017 and 2012) during the short three-month growing season, which represents one percent of the state's total agricultural commodities (USDA NASS, 2017). Recent farming trends have revealed a twofold increase in the number of farms producing squash in Montana from 1997 to 2017 (2002 USDA Census of Agriculture, 2017 USDA Census of Agriculture), potentially due to the crops low production and labor cost. About a quarter of vegetable farmers in the state are growing winter squash (USDA NASS, 2017 and 2012) and many of them do not fully understand the best way to cure or store winter squash in our cool, semi-arid climate (D. Blankenbaker, Personal Communication, October 25th, 2021).

Winter squash is part of the *Cucurbita* genus and can be divided into categories by three of its most prominent species: *maxima*, *moschata*, and *pepo*. What differentiates these species is where they are thought to have originated from, and their physical characteristics. The cultivated species from the genus *Cucurbita* were all domesticated in different regions across North and South America during the pre-Columbian era, with *C. pepo* having the earliest documentation of domestication around 8,000 years ago (Ferriol, 2008). While growing, the *C. pepo* varieties tend

to have smaller fruits and shorter vines, varieties belonging to *C. moschata* tolerate higher temperatures and pest pressure well, and those of *C. maxima* tend to have larger fruits with longer vines. In storage, *C. maxima* varieties develop higher levels of sweetness, *C. moschata* varieties experience a decrease in quality at a more linear rate, and *C. pepo* varieties tend to ripen sooner.

The response of winter squash to its production and storage environment is unique to each variety. For example, kabocha and buttercup types (*C. maxima*) are known to require longer periods of time in storage when compared to other varieties (Wetzel 2018, Loy 2010). Because of this, with time their flesh becomes sweeter and develops a pastier texture which is preferred in some markets (Hurst, 1995). Butternut varieties (*C. moschata*) can tolerate higher levels of heat and humidity during their production stage while displaying a greater resistance to disease and insects, and they store well for a few months (Ferriol, 2008). Acorn and Spaghetti types of squash (*C. pepo*) tend to be smaller in size and more prolific producers but don't offer the same level of sweetness or texture when compared to kabochas, and don't store as long (Loy, 2010). In comparison, according to Dr. Brent Loy who was a researcher from The University of New Hampshire and devoted his life to the study of acorn squash, delicata type squash (which are also a *pepo* variety) may be a better choice for consumers looking for an earlier ripening sweet winter squash (Loy, n.d).

Providing ideal temperature and relative humidity (RH) levels after harvest keeps the flesh of the fruit firm, prevents rot, and encourages the development of sugars which gives squash its pleasing taste (Harvey et al., 1997). Each winter squash variety has its own harvest and storage requirements, and when done correctly, they help produce a desirable product that

consumers could access for most of the year (Wetzel, 2018). However, current sales trends suggest locally produced winter squash is only readily available to consumers from October through January (United States Department of Agriculture 2017; USITC Census 2021). If squash is only being stored and sold for up to three months after harvest, we are missing out on a 100 percent increase in sales of specific varieties to consumers who have expressed an interest in purchasing this product and enjoying its benefits throughout the year.

Bozeman Montana has, on average, 95 freeze free days at a 50 percent probability level (Montana State University Extension, 2023) which means it can be challenging to mature even one full crop of winter squash per year. However, the drier air and lower soil moisture levels of this region allows for lower pathogen populations (Babadoost et al, 2009) and the rocky mountain range protects us from pests like the cucumber beetle, squash vine borer, and squash bug (Haber et al 2021). Since soil-borne pathogens can cause squash to rot while in storage and insects can damage squash during the production phase, Montana growers may face fewer pest and disease pressures when it comes to winter squash production and storage.

Because winter squash is not frost tolerant, in Montana the entire year's supply is planted in early summer and harvested in September before being stored for eating from October until the supply diminishes. Bozeman vegetable-farmer Nate Brown has increased his production rates of squash consistently over the last three years, and still manages to sell all his winter squash by the end of December. Harlequin farms in Polson, MT utilizes a large pack shed to store their winter squash through January, but the propane that is required to heat this space during the cold months is an additional cost. Max, a successful farmer south of Missoula, is transitioning to a winter only CSA program on his farm, where winter squash will be made available to his

customers through March. In the same farming region, another farmer stopped growing winter squash all together because the pressure to harvest on time and find adequate storage was too stressful. Currently, general storage recommendations for winter squash exist, but the specifications offered by extension agents across the United States vary regarding how long each variety will store and what temperature or level of RH is best. This variation may be due to squash storage outcomes being influenced by regional climate trends.

Research that has focused on winter squash has uncovered significant intraspecies variability among the *Cucurbita* genus and consistent intrinsic differences in composition among variety and level of maturity in any given variety. The significant differences found among cultivars in nutrient compositions, biochemical properties, and sensory evaluations (Culpepper and Moon, 1945; Corrigan et al 2006; Kostecka-Gugała , 2020) may be explained by high genetic variability due to natural hybridization between populations of this species. However, Culpepper and Moon argue that “for most constituents, the age of the fruit is the source of the greater variation than variety. There is considerable unexplained variability, obviously due to sampling error, resulting from the large differences in individual fruits.” It should be noted that storage temperatures during these trials were between 40- and 50-degrees Fahrenheit which is cooler than ideal and may cause chilling injury.

Most of the winter squash research that has occurred over the last 30 years has focused solely on the *maxima* species, specifically buttercup varieties which are closely related to kabochas. This is because there is substantial demand for them in Japan, which creates a market opportunity for Mexico and New Zealand to fill (Wright and Grant 2010, Harvey 1992). The high demand for buttercup and kabocha varieties is due to the higher levels of sweetness and

pasty texture that is deemed more desirable (Corrigan 2000, Harvey 1997). Researchers in the United States have also begun to invest their time and attention into buttercup and kabocha varieties because of their storability and nutritional value (Wetzel 2018). However, research that has focused on *C. pepo* or *C. moschata* is lacking. There were a handful of more comprehensive studies in the United States from 1945-1965 (Culpepper and Moon 1945; Merrow and Hopp 1961; Schales and Isenberg 1963) that included all three species, however more data is needed on changes in biochemical attributes during fruit development and storage in these varieties.

Based on the research that has been done so far, we know that while winter squash is being stored, starch decreases over time as it is converted to sugar by enzymes. This conversion causes an increase in soluble sugar content until the starch is depleted, at which time the sugars then decrease. The soluble sugar content (SSC) is the dissolved sugar or solids within an aqueous solution and is the measurement (°Brix) taken by a refractometer to determine sweetness. Dry matter decreases gradually with time, and the change in interior flesh color varies by variety but usually becomes darker or redder over time. The rate of change in the mass of winter squash is related to the temperature and time spent in storage (Wright and Grant 1999); as time in storage increases weight loss increases and as temperatures increase, weight loss increases (Wright and Grant 1999, Nagao 1991). However, since most of this research has taken place in subtropical or sub humid regions, it is not applicable to semi-arid and cooler regions like Montana which was one motivation for our research.

Increasing our knowledge about specific storage requirements for each variety of winter squash in a cool, semi-arid climate will present opportunities for expanding local markets and increased consumption by extending availability past December and into March. With this

knowledge, producers and retailers could plan their squash sales accordingly by selling varieties that don't store as well sooner in the season and selling longer storing varieties later. This change will lead to an increased availability of a locally grown, nutrient dense vegetable. Analyzing the storage ability of winter squash varieties in Montana will allow us to improve local storage methods and meet the increase in consumer demand, while improving our local market capacity.

Storage Environment and its Relationship to Fruit Quality:

Although regional variation exists, the current industry standard for recommendations on temperature for winter squash storage is 50-55 degrees Fahrenheit (Formiga, et al., 2019) (Harvey, 1997) (Post Harvest Technology Center, UC Davis, 1998). When storage temperatures exceed 59 degrees, squash become discolored, and the texture of the flesh becomes stringy. The current recommendations for RH within a storage facility are between 50 and 70. Higher RH levels promote decay, and lower levels cause excess weight loss and texture deterioration; lower RH minimizes decay (Brecht, 2004). This research aims to determine how and in what way the storage environment affects varieties of squash differently in a cool semi-arid region. Harvesting schedules and post-harvest handling among winter squash species may vary considerably, including how their flesh responds to RH and temperature, and how that affects eating quality (Loy, 2010).

This study will explore how two different storage environments affect the fruit quality of 10 different winter squash varieties. We will also identify short and long storing varieties of winter squash by comparing current recommendations of cold storage to an uncontrolled environment that may more closely represent what many squash farmers should have access to. Specifically, we will test whether the varieties of winter squash grown at the Bozeman

Agricultural Research and Teaching farm can be stored for six months without exhibiting dehydration, discoloration, or decay. Examining how storage affects the quality of different types of winter squash will allow producers, farmers, restaurant owners, wholesalers, and individual consumers to better understand the value associated with nutritional composition of cultivars that they may choose to grow or purchase for sale. Understanding the value of nutritional composition will allow consumers to make better informed decisions about the type of squash they would purchase in store or what they would like to grow themselves. Looking at the length of time that each variety stores for will help us decrease storage loss and improve our local food supply infrastructure.

Fruit Quality and Nutrient Composition:

The culinary quality of winter squash is important to consumers who value incorporating a healthy, well-balanced diet into their lifestyle. Many consumers now consider integrating a healthy, well-balanced diet a necessary aspect of their lifestyle. As people continue to gain knowledge about the benefits of a well-rounded diet, the demand for locally sourced nutrient-dense fruits and vegetables will continue to increase (Low, 2015). Additionally, it is more likely that consumers will purchase higher quality squash consistently. (Noseworthy, ?). There are dozens of winter squash varieties available and each one of them is known for its unique taste, texture, appearance, and numerous health benefits. Winter squash are known for their bold yellow, orange, or red flesh. These bright colors are the result of pigmented compounds known as carotenes which are precursors to vitamin A. Vitamin A helps maintain a healthy immune system, plays a crucial role in vision, and encourages overall health (Seo et al., 2005). When compared to sweet potatoes, winter squash is a low caloric food with a low glycemic index and

load, and the polysaccharides they contain help regulate and control blood sugar and cholesterol. They are also rich in potassium which benefits the heart by counteracting the harmful effects of sodium on blood pressure (Harvard School of Public Health, 2023).

Nutrition and taste are not the only attractive qualities that winter squash exhibit. They are also known for their ability to store well over long periods of time given the right conditions. In other regions of the United States, and the world, extensive research has shown that under the right conditions certain varieties of winter squash can be stored for over six months while maintaining fruit structure, taste, and nutritional qualities (Kami et al., 2011). The ability to store nutrient dense foods for long periods of time is beneficial to communities in rural areas, however it would also help to decrease the effects of unnecessary food miles and reduce our dependence on a shifting supply chain.

Price and affordability are also determining factors for most consumers when choosing what produce to purchase. Taste, texture, and nutrient composition are characteristics that both consumers and producers should be aware of. However, the producer's greatest concern is often yield, followed by appearance, and then storage efficiency.

Quality standards for winter squash that have been set by the USDA are vague and insufficient. The grades assigned to them relate to the exterior appearance of the fruit instead of eating quality of the flesh (USDA Fall and Winter Type Squash and Pumpkin Grades and Standards, 2023). The quality of winter squash is best determined by its nutrient composition. Sugar, starch, and fiber are the major components that contribute to taste and texture. Each type of winter squash has a distinct nutritional composition. The size of winter squash and the number of fruits each plant produces also vary by variety. The quantity or amount each winter squash

variety produces is associated with yield. It is important for both consumers and producers to understand that when it comes to winter squash production, quantity does not equal quality.

The nutritional composition of winter squash contributes to the fruit's weight or density. The moisture content of squash contributes to yield and quantity which can make it a valuable characteristic to some farmers. Moisture content also contributes to the texture and mouthfeel of squash. Dry (DM) matter content of the mesocarp is one of the parameters most closely related to flesh quality in winter squash. DM dictates how much moisture is contained within the flesh and is determined by the amount of starch content in the fruit's flesh, which is gradually converted to sugar over time (Ferriol, 2008). DM is a percentage measured by dividing a unit's dry weight by the total initial weight of the same sample. In other words, it is the solid portion that remains after all the moisture has been removed from the squash.

Sugar and starch are both carbohydrates and together they make up 50-70% of a squash's DM. Varieties with a higher DM content have a better eating quality than squash with low DM because additional starch contributes to a pasty texture when cooked (Loy, 2010). This pasty texture has been shown to be more desirable in sensory attributes that were evaluated in cooked squash sensory evaluations (Harvey 1997, Lacuzzo et al 2008; Corrigan 2010). During post-harvest storage, starch is converted into sugar which results in improved flavor and sweetness. Carotenoid levels also increase during this time (Conti 2014, Lacuzzo, 2008).

When considering eating quality, both high SSC content and richer color (provided by carotenoids) are strongly associated with high consumer ratings of eating quality in winter squash (Wyatt 2016, Wright and Grant 1999, Schales, and Isenberg 1963). Since starch is converted to sugar during storage, SSC will continue to increase if starch is still present. As

storage time increases, starch decreases. Once starch has been depleted, sugars are no longer able to increase and water content makes up a greater portion of the flesh. A less pasty or waterier flesh is considered less appealing. This happens sooner in varieties that have a lower DM to begin with.

In Brent Loy's paper "A Growers Paradox" (Loy 2010), he compares varieties of squash that have different levels of DM. He states that winter squash with a lower percentage of DM (<10%) are typically perceived as having a lower culinary quality, especially when compared to varieties that have a percentage DM above 20%. Varieties of winter squash that have a higher percentage of DM also have a higher percentage of starch in their flesh. Once harvested, as starch is converted into sugar over time, the increase in sugars is primarily the result of the additional starch as a storage reserve. However, since varieties with a lower percentage of DM have a greater percentage of water present in the mesocarp, their fresh weight yield is higher. For example, varieties that have a lower percentage of DM (10% for example) will produce twice the fresh weight yield as a variety with 20% DM would have, since water weighs more than the constituents of squash flesh. Loy concludes that because of this, if a grower is interested in marketing fruit that is known for its fruit quality, they often end up sacrificing fresh weight yield which leads to reduced profit (Loy, 2010). In most acorn and spaghetti types, the conversion of starch to sugar occurs earlier in fruit development meaning the ideal SSC may be attainable much earlier, and producers would not *need* to wait for SS to reach the desired content. The benefit here for growers is a product that is ready to sell earlier in the season without the cost of storage, however consumers may be missing out on some of the culinary and nutritional qualities that other varieties can offer after a few months in storage later in the season. Alternatively,

butternut squash requires at least 60 days of storage to develop adequate SSC (Loy 2010, Conti 2015, Lacuzzo 2009, Culpepper & Moon 1945) and may offer the consumer better taste and texture. Kabocha buttercup squash produce fewer fruit per plant on average when compared to butternut and acorn squash types (Formiga 2019, Wetzal 2019) and store longer, resulting in the attainment of higher SSC and carotenoids over time which consumers find appealing.

Producers and consumers often use the term “ripe” to describe when a fruit or vegetable is ready for consumption. Ripeness is usually attained once a fruit or vegetable has fully matured and is deemed ready for consumption. Winter squash are unique in that its perception and definition of ripeness varies among researchers. Noseworthy suggests that “the term ‘ripening’ may not be appropriate for squash because it implies a stage of maturity has been reached where it can be consumed”, yet some squash require weeks or even months of storage before they should be enjoyed (Harvey 1997, Noseworthy 2012). From a physiology perspective, there are three stages of fruit development in winter squash: fruit growth and expansion (20-30 days after pollination), dry matter accumulation (30-40 days after pollination), and maturation (40-60 days after pollination). During maturation embryo growth, seed fill, starch degradation, and sucrose accumulation are all occurring simultaneously (Loy, 2010)

When time allows, growers often determine their harvest date by a darkening in color of the ground spot or by observing fruit set and scheduling harvest between 50 and 60 days after that event. However, butternut squash usually requires more than 10 days for flower and fruit set, so this is not a reliable method in that scenario. Leaf senescence and the measuring of rind hardness seem to be reliable indicators for a harvest date but do not indicate the levels of SS, DM, or starch of the squash’s flesh (Loy 2005, Noseworthy 2012).

CHAPTER TWO

THE EFFECTS OF STORAGE ENVIRONMENT, TIME, AND VARIETY ON WINTER
SQUASH QUALITYMaterials and Methods

For this study, we chose winter squash varieties that are already known to store well as well as some varieties that have performed variably under different environmental circumstances, and varieties that have very limited data available. We examined ten winter squash varieties, each with unique physical properties, taste, texture, and nutritional composition. These variations in characteristics are determined by both genetics and by the environment in which they are grown and stored. Growers can manage the taste, texture, and color of squash flesh by providing an optimal storage environment for them.

Site Description.

Bozeman Montana is located within the Gallatin Valley which is in the heart of the Rocky Mountains. Field trials were located at Montana State University's Bozeman Agriculture Research and Teaching (BART) farm in Bozeman, Montana on a Turner silty clay loam at 45.6663 degrees north, 111.0696 degrees west, and 1,500 m elevation. The growing season typically begins in June when temperatures are likely to remain above freezing. The average low temperature in June is 44 degrees Fahrenheit, while the average high temperature is 73 degrees; the season usually ends by the beginning of October when temperatures begin to dip into the 30s once again. The average precipitation for Bozeman in June is 2.78 inches, and between 1.2 and 1.4 inches for the months of July through September (table 1) making irrigation necessary for

any vegetable producer in the region. Most farmers use their pack shed building and any coolers that become empty during the winter months for winter squash storage. Squash is stored in large palletted bins that are stacked on top of each other. Typically, growers in this area try to sell all their squash by December 1st to avoid loss of product.

Month	Average High Temperature	Average Low Temperature	Precipitation (inches in rain)	Precipitation (inches in snow)
January	34	13	0.5	9
February	38	16	0.5	6
March	46	23	1	8
April	56	30	1.7	4
May	65	38	2.7	1
June	73	44	2.7	0
July	83	50	1.3	0
August	82	48	1.3	0
September	72	41	1.5	0
October	58	32	1.4	3
November	43	21	0.9	8
December	34	13	0.6	11

Table 1: Average Temperature and Precipitation by Month in Bozeman, MT.

Crop Planting and Management

We started seeds in a greenhouse that was maintained at 72 degrees Fahrenheit during the day, and 65 at night (+/- 7 degrees) with a 16-hour photoperiod on 05/15/22. The seedlings were planted in the field between June 4th and June 7th, 2022, to decrease the risk of frost injury.

Table 2 contains descriptions of the squash grown and stored in trials during the summer and winter of 2022-2023 in Bozeman, MT. The ‘Starry Night’ acorn, ‘Pinnacle’ spaghetti, ‘Tetsukabuto’, ‘Metro PMR’ butternut, ‘North Georgia Candy Roaster’, ‘Sunshine’ kabocha, ‘Red Kuri’, and ‘Winter Sweet’ kabocha seeds were purchased from Johnny's Seeds (Albion, Maine). The ‘Honey Boat’ delicata seeds were purchased from High Mowing Seeds (Wolcott,

Vermont) and the ‘Lower Salmon River’ seeds were purchased from Adaptive Seeds (Sweet Home, Oregon).

Species	Type	Variety	Hybrid Status	Days to Maturity	Vining Habit	Fruit Size (lbs.)	Storage Length
<i>C. Pepo</i>	acorn	Starry Night	F1	95	Bush	2-2.5	Mid
	delicata	Honey Boat	F1	100	Long	1-1.5	Mid
	spaghetti	Pinnacle	F1	85	Semi Bush	2-2.5	Early
<i>C. Maxima x Moschata Hybrid</i>	kabocha butternut hybrid	Tetsukabuto	F1	100	Long	2.0-3.0	Late
<i>C. Moschata</i>	butternut	Metro PMR	F1	105	Medium	2-2.5	Late
<i>C. Maxima</i>	hubbard	NGCR	OP	100	Long	5-7.5	Late
	hubbard	Red Kuri	OP	92	Long	1.5-2	Mid
	buttercup	LSR	OP	90	Long	5-6.5	Late
	kabocha	Winter Sweet	F1	95	Long	3-3.5	Late
	kabocha	Sunshine	F1	95	Long	3-3.5	Late

Table 2: Winter Squash grown and stored in our 2022-2023 trial by Species, including information on days to maturity and fruit size.

All seeds were planted into 50 cell flats with OMRI approved Sunshine mix (no. 4) growing media. The flats were watered as needed and fertilized with 5-1-1 Alaska Fish fertilizer as needed. Plants were transplanted at two weeks and planted into beds covered by black plastic

mulch to protect against cold temperatures, suppress weeds, and conserve soil moisture. The plots were irrigated using drip tape irrigation to reduce both water use and fungal disease. Weeds were controlled by cultivation and hand weeding. Insect and disease pressure was monitored, and no protective treatments were applied or needed. Squash plants were grown in single rows, in ten separate beds that were 400 feet long. Row spacing was five feet and in-row spacing was 2.0 feet for *C. pepo* varieties and 3.0 feet for the others, which follows existing planting suggestions by extension agents. Table three details the differences in yield per variety that were grown for this trial.

Variety	Plants	Fruits	Yield	Fruit/plant	Fruit Weight	Yield per Plant
Metro PMR	129	452	917	3.50	2.03	7.12
Honey Boat	215	734	829	3.42	1.13	3.86
Winter Sweet	120	212	698	1.77	3.29	5.82
NGCR	128	203	1218	1.59	6.00	9.54
Pinnacle	200	625	1395	3.13	2.23	6.98
LSR	126	182	895	1.12	4.92	5.50
Sunshine	143	279	864	1.95	3.10	6.05
Starry Night	245	417	893	1.70	2.14	3.64
Red Kuri	122	211	397	1.73	1.88	3.25
Tetsukabuto	218	561	1422	2.57	2.53	6.50

Table 3: Winter squash production and harvest data from our 2022 growing season in Bozeman, MT. Measurements are per bed. Individual fruit weight is averaged, and yield (per bed) is measured in pounds.

Sainfoin (*Onobrychis viciifolia*) had been growing in this plot for four consecutive years leading up to this experiment. A soil test taken at the end of July revealed that all nutrients tested were above sufficiency levels except Nitrate N (13 lb. per acre) and Chloride (7 lb. per acre). Since both of these nutrients are known to leach, they are often found at higher levels deeper than the 6" depth which was sampled. The soil pH was 7.3. Our fruit weight and fruit per plant yields

(table 3) fell within the range listed on their seed packets (table 2). Plants appeared healthy and growth was vigorous throughout the season.

Squash Harvest

Once the squash reached maturity and the danger of a hard frost was present, we harvested the fruit by hand by using a clipper to cut the vine about two inches from the fruit on September 20th, 2022. Then, each variety of squash was placed into a large bin before being brought into the barn where we sorted and placed them into their assigned storage units. Due to forecasted temperatures of below freezing, we did not cure our squash. Storage began on September 22nd, analysis began on October 22nd and continued monthly through March.

Storage Environments

Two storage environments were used to hold squash from October through March. Storage A is an insulated walk-in storage shed inside of a large barn structure that closely resembles what a farmer in this area may have access to. RH was measured but not maintained. A heating unit was placed inside the shed and the temperature was monitored to ensure that it was maintained above freezing. Storage B is a controlled walk-in cooler in the plant growth center on campus, with the temperature held at 53 degrees F. RH levels were measured but not controlled. Each month unmarketable fruit were counted, recorded, and removed from collection. Temperature and RH levels were recorded with a Campbell Scientific CR 300 data logger and CS215 digital air temperature and relative humidity sensor in each storage environment.

Month	Barn Temp(A)	Barn RH (A)	Cooler Temp (B)	Cooler RH (B)
1	55.4	46	51.6	78.2
2	58.8	26.4	51.6	72.6
3	59.3	19.9	51.6	67.6
4	60.8	21.5	51.4	64.9
5	61	19.4	51.3	61.1
6	58.1	22.2	50.9	58.7

Table 4: Mean temperature and relative humidity of storage environments per month, of uncontrolled barn and controlled cooler.

Squash Varieties

Ten cultivars of winter squash (Table 2) were chosen for this project so that we could demonstrate culinary and storage potential among the winter squash cultivars that local farmers currently produce.

Experimental Design

We used a completely randomized design (CRD) to assign treatments of storage time to each winter squash. The treatment was replicated for each storage environment. The treatment levels are categorical and are implemented as different amounts of time: one month, two months, three months, four months, five months, and six months after harvest. There were 10 varieties of squash being analyzed and four replications of each variety were analyzed per month, in each storage environment. Each treatment level of storage time was assigned to an individual squash through a random number, and then the random numbers were sorted in order so that we could produce a random application order of time treatments for each variety of the squash harvested. The squash was then randomly distributed into storage bins so that an equal number of fruits from each cultivar was selected for each storage treatment. Each bin contained six fruits. Bins were vented to encourage air circulation and stackable, at 16”x24”x8” tall.

Chemical Composition Measurements

For six months, sugars, starch, exterior skin color, interior flesh color, and dry matter measurements were taken from four of each squash variety in each storage environment on a monthly basis. Sweetness, measured as soluble sugar content (SSC, °Brix) was determined by a sample of squash juice (manually obtained) with a Milwaukee instruments ma 871 refractometer. We measured how the levels of starch changed over time with a starch assay adapted from Nature Protocols (Smith and Zeeman. 2006.1:1342-1345). The change in color of squash was measured using a Minolta CR-20 Color Meter (Konica Minolta Sensing Americas, Ramsey, NJ). Three readings of the parameters L*, a*, and b* were taken from each sample at 120° apart. Dry matter was calculated by placing a 15 g sample of squash into a paper bag and leaving it to dry in an oven at 110 degrees F. Once the sample had dried completely, its weight was measured again and divided by the original weight to find the percentage of dry matter.

We also calculated how the mass of each squash variety changed over time by measuring the weight of the same five winter squash that had been set aside from each variety, each month, in both storage environments. These squashes were different units than the squash being measured for their chemical composition but were stored in the same storage environments. Measuring the changes in SSC, color, dry matter, starch, and mass of each variety have given us insight into how different cultivars of winter squash perform while being stored in a semiarid climate with a short growing season.

Dry Matter: Percentage dry matter (DM) was calculated from the ratio of oven-dried and fresh weights.

Starch: A metal borer was used to remove 2 samples from each variety of squash. The samples were then frozen with liquid nitrogen before being ground into a fine powder for the starch

assay. The starch assay protocol adapted by Nature Protocols (Smith and Zeeman. 2006.1:1342-1345) was adjusted so that it could be used on tissue with a higher proportion of starch.

Brix: From each fruit, two samples (c. 20 g each) of mesocarp, free of seeds, placenta, and skin, were obtained from segments taken from opposite sides, taking care to avoid the ground spot.

These samples were frozen for 24 hours, and then thawed for an easier extraction of juice from the flesh. Once manually extracted, the juice was placed on a refractometer to measure Brix.

Color (interior): The fruit was cut in half longitudinally with three readings of reflectance taken directly onto the flesh between the stalk and base of the fruit, excluding the ground spot, the skin, and seed area. The mean of the three readings taken with a Konika Minolta CR-20 Color Reader gives the color of the flesh in CIE $L^*a^*b^*$ notation where L = lightness increasing from dark to light, a^* = color tone changing from bluish green to red purple, and b^* = color tone changing from blue to yellow.

Mass: Mass was measured by weighing (in grams) the same five fruits of each variety being stored per storage environment monthly.

Statistical Analysis

All statistical analyses were performed using R Studio 2022.02.1+461 "Prairie Trillium" Release for macOS. Differences in DM content (%), starch content (mg/g dry weight), SSC ($^{\circ}$ Brix), mass (grams fresh weight), and color (CIE $L^*a^*b^*$ values) were examined using ANOVA. Significant differences determined by a Post Hoc multiple comparisons of means using LSD with a 95% family-wise confidence level.

Results and Discussion: The Effects of Cultivar, Time, and Storage

Results

A completely randomized design was used to assign treatments of 10 different cultivars, six different time periods, and two different storage environments. In our analysis of DM, starch, and brix content there was a greater difference in means among cultivars and storage time than among storage environments. In mass and color, the cultivar and date were equally significant and always more significant than storage.

Dry Matter

There was a simple effect of cultivar ($P < 2.2e-16$) which is shown in table 5, and no significant interaction between storage environment, variety, and storage time.

Cultivar	Storage Period:	1 Month	2 Months	3 Months	4 Months	5 Months	6 Months
<u>% Dry Matter ($P < .05$)</u>							
Winter Sweet		30.19	26.07	26.99	25.93	24.55	24.73
Starry Night		24.56	23.16	22.49	20.99	22.56	21.52
Tetsukabuto		19.80	18.64	18.36	17.84	18.23	17.48
Honey Boat		18.29	16.82	15.28	13.28	13.77	14.75
Sunshine		17.60	18.69	16.79	17.88	14.70	14.94
Red Kuri		16.64	15.05	14.76	12.21	12.12	12.93
NGCR		14.71	14.14	13.84	12.69	12.45	11.32
Metro PMR		12.73	13.70	13.45	13.59	13.93	12.30
LSR		11.25	10.62	10.38	11.68	11.75	12.16
Pinnacle		7.07	7.07	6.36	6.41	6.39	6.07

Table 5: Means of dry matter content across both storage environments after being stored for 1, 2, 3, 4, 5, or 6 months. NGCR= North Georgia Candy Roaster. LSR= Lower Salmon River.

There was a gradual decrease in all cultivars over time (figure 1).

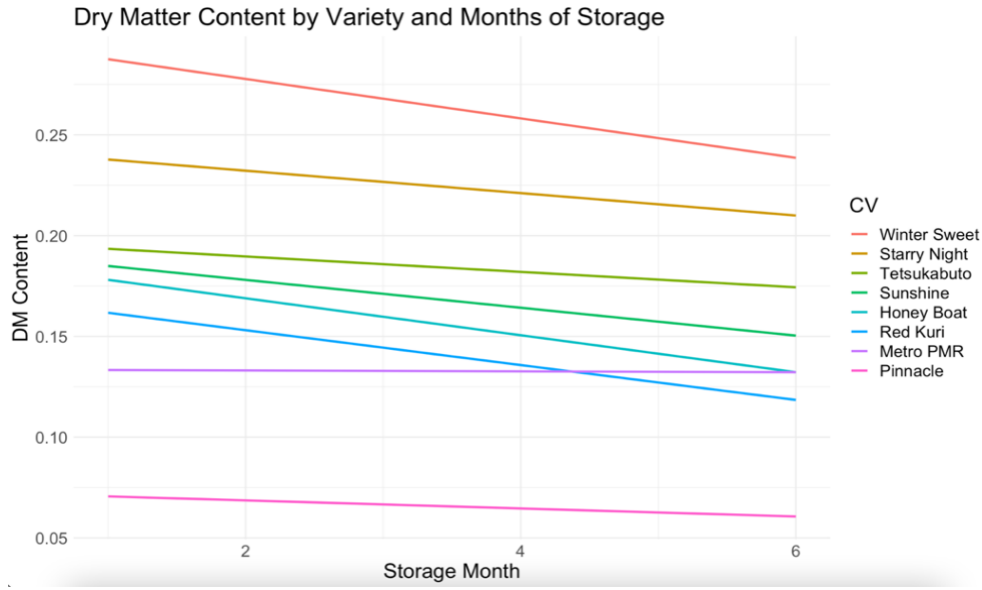


Figure 1: Mean DM content of each variety gradually decreasing over time in both storage environments.

In cultivars with a higher percentage of DM and starch to begin with, the higher levels of starch led to a greater gain in sugar later (table 6).

Variety	DM	Starch	Soluble Solids
Winter Sweet	0.26a	528.55a	14.2b
Tetsukabuto	0.18c	362.06b	10.58c
Starry Night	0.22b	289.82c	17.03a
Metro PMR	0.13f	258.65cd	9.75d
Red Kuri	0.14f	240.88cde	8.99ef
Sunshine	0.17d	236.51de	11.97c
NGCR	0.13f	197.39ef	9.11e
Honey Boat	0.16e	160.4f	12.11f
LSR	0.11g	91.48g	7.92g
Pinnacle	0.07h	14.8h	4.82h

Table 6: Percent DM, Starch ($\mu\text{g}\cdot\text{g}^{-1}$ DW), and Soluble Solids (g per 100 grams of solution) between varieties tested. Significant differences determined by a Post Hoc multiple comparisons of means using LSD with a 95% family-wise confidence level. LSR= Lower Salmon River. NGCR= North Georgia Candy Roaster.

Starch Content

We saw a simple effect of cultivar ($P < 2.2e-16$) and an interaction between time and cultivar with our starch analysis (figure 2). Starch decreased over time in each of the varieties we analyzed (Table 7), however the rate at which starch is converted into sugar varies by variety and is likely dependent on the amount of DM at harvest. In varieties with a greater amount of DM to start with, there is a greater amount of starch that can be used by enzymes to convert carbohydrates to sugar. Table 6 illustrates how the higher average starch levels of ‘Winter Sweet’, ‘Tetsukabuto’, and ‘Starry Night’ lead to a higher SSC in the same varieties, when compared to others that were analyzed.

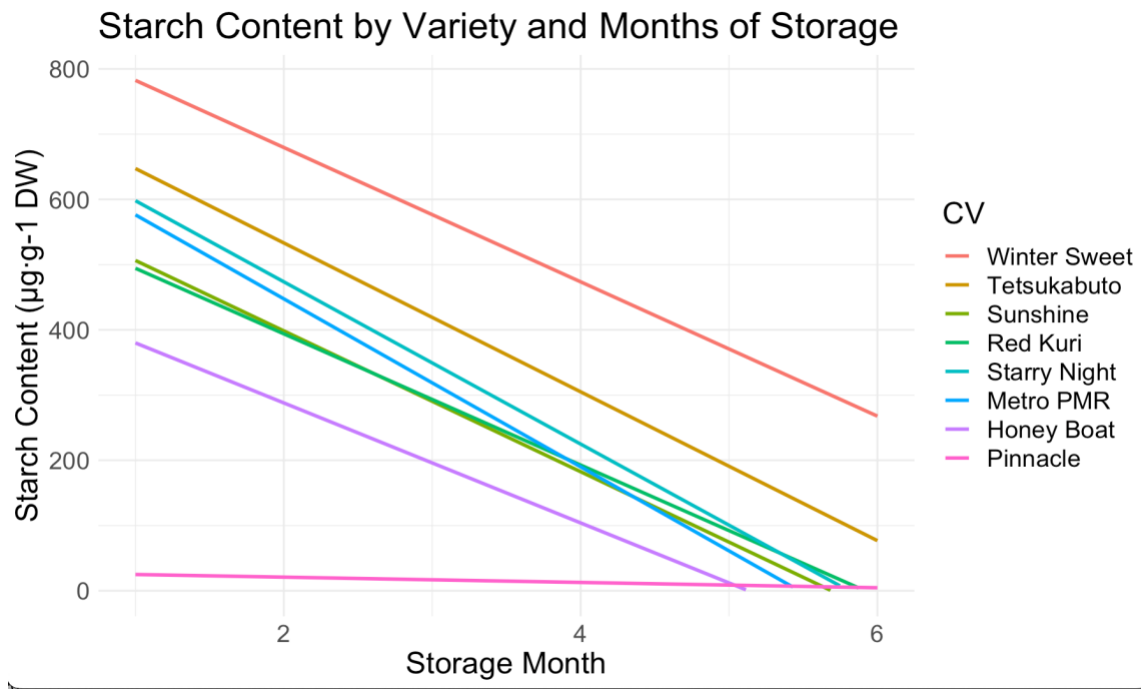


Figure 2: Starch content of each variety, averaged over both storage environments, decreasing at different rates over the 6 months of storage.

Cultivar	Storage Period:	1 Month	2 Months	3 Months	4 Months	5 Months	6 Months
Starch (mg·g DW)							
Winter Sweet		654.7	729.5	608.7	657.5	408.7	123.4
Tetsukabuto		723.8	426.6	408.5	328.3	220.1	65
Starry Night		690.4	420.2	282.3	188.5	108.1	24.1
Sunshine		580.4	372.2	197.7	196.2	60.3	12.3
Metro PMR		712.1	359.7	199.6	196.1	26.6	12.5
Red Kuri		618.6	291.3	259.1	111.8	74.9	79.8
NGCR		638.7	244.7	121.1	79.8	57.4	10.4
Honey Boat		541.2	181.5	24.5	35.7	3.8	1.8
LSR		356.1	84.1	40.9	21.2	17.1	11.4
Pinnacle		19.9	23.2	23.4	10.4	9.6	2.3

Table 7: Means of starch content across both storage environments after being stored for 1, 2, 3, 4, 5, or 6 months. NGCR= North Georgia Candy Roaster. LSR= Lower Salmon River.

On average, 'Winter Sweet' had the highest levels of starch. During the second month of storage, it peaked at 730 mg/g, and by the end of our storage trial its starch levels were still higher than any other variety (250 mg/g). 'Tetsukabuto' averaged just over 720 mg/g one month after harvest, while 'Starry Night' and 'Metro PMR' also started out near 700 mg/g to begin with. However, their starch contents dropped off more quickly than 'Winter Sweet' did. The starch content of the 'Starry Night' (108 mg/g) was less than half of that of 'Tetsukabuto' (220 mg/g) by the fifth month, and 'Metro PMR' (26.6 mg/g) was one quarter of 'Starry Night' during the same time frame. 'Red Kuri' and 'Sunshine' started out at around 600 mg/g, but their starch reserves were depleted more quickly than those previously mentioned. The 'Honey Boat' started with a starch content of around 541 mg/g and 'Pinnacle' was close to zero (Table 7). 'Winter Sweet' and 'Tetsukabuto' had higher levels of starch at harvest in comparison to the other cultivars, and it lasted through the end of our trial.

Soluble Solids Content

There was a highly significant effect of cultivar on SSC ($P < 2.2e-16$) and a significant interaction that occurred between cultivar and storage time (figure 3) on SSC ($P 1.384e-11$). Some varieties increased in soluble solids while in storage, while others decreased. The 'Starry Night' variety had the highest amount of SS after harvest, averaging 17.03g, and then increasing to 17.5 g through the sixth month of storage. The 'Winter Sweet' variety started around 12.5 g and also increased to 16 g. The 'Testukabuto' started with an average SSC of 10 g and increased to 11.5 g, similarly the 'Metro PMR' variety increased over time, but at a lower and slower rate (Table 8).

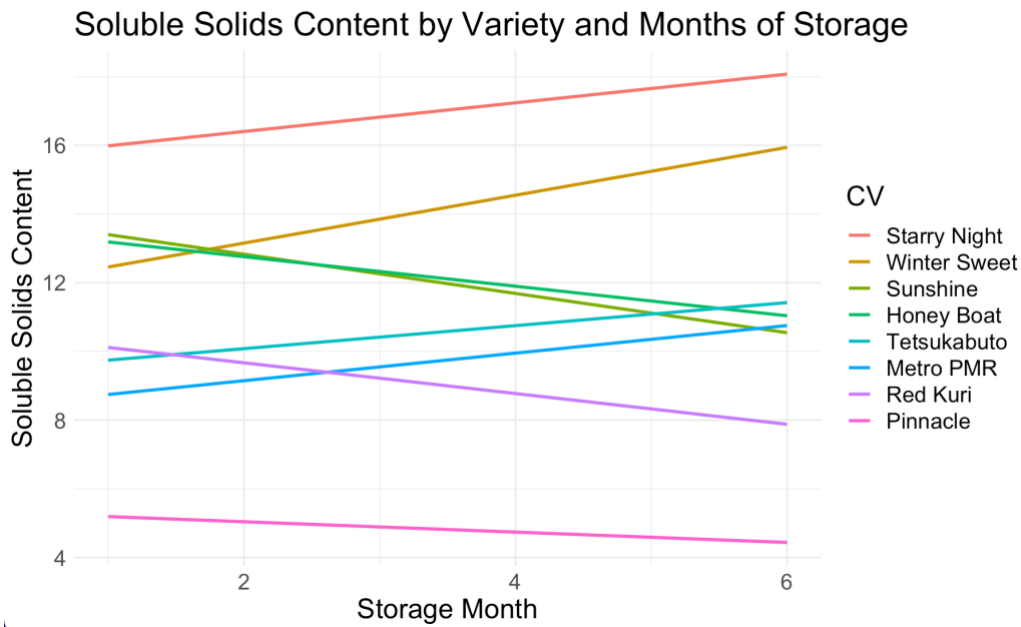


Figure 3: Soluble solids content of each variety, averaged over both storage environments over the 6 months of storage. Starry Night and Winter Sweet increase, while Sunshine, Honey Boat, and Red Kuri decrease.

The 'Honey Boat', 'Red Kuri', and 'Sunshine' varieties had SS levels that averaged as a loss over time. 'Honey Boat' and 'Sunshine' averaged at 12.5g after harvest and decreased to

about 10 g by the sixth month of storage. 'Red Kuri' started around 10g and dropped to around 7.5 g by the sixth month of storage. 'Pinnacle' averaged less than 5 g of soluble solids throughout the storage duration (Table 8).

Cultivar	Storage Period:	1 Month	2 Months	3 Months	4 Months	5 Months	6 Months
<u>Soluble Sugar (g)</u>							
Starry Night		15.60	17.19	17.55	15.98	18.60	18.18
Winter Sweet		11.63	12.30	15.57	15.31	15.33	14.81
Honey Boat		13.03	13.38	12.53	9.50	11.85	12.18
Tetsukabuto		9.58	9.70	10.65	11.19	11.89	10.50
Sunshine		12.71	13.20	12.29	12.60	11.16	9.88
Metro PMR		8.09	9.53	9.58	10.19	11.70	9.46
Red Kuri		9.89	9.86	9.90	8.07	7.91	8.24
LSR		7.89	7.53	7.90	8.19	7.97	8.21
NGCR		8.84	10.10	10.14	9.03	9.24	7.54
Pinnacle		5.29	5.23	4.70	4.23	5.00	4.46

Table 8: Means of soluble solids content across both storage environments after being stored for 1, 2, 3, 4, 5, or 6 months. NGCR= North Georgia Candy Roaster. LSR= Lower Salmon River.

Regarding the rates in which SSC changed, when the SSC of a cultivar increased over time, it likely was a variety that had more starch to begin with. Additionally, if the starch content was higher at harvest, it lasted longer through storage. 'Starry Night PMR' and 'Winter Sweet' varieties have more starch and therefore, their SS's do not average as a loss. To a lesser extent, the SS content of Metro PMR' and 'Tetsukabuto' also averaged an increase in comparison to the levels they started at (Figure 3).

Mass

More mass was lost in the uncontrolled barn unit, specifically during the first month of storage (Figure 4). There was also a simple effect of time ($P = 5.443 \times 10^{-13}$) on the mass of winter squash.

All squash lose weight in storage and the total amount lost is unique to each variety.

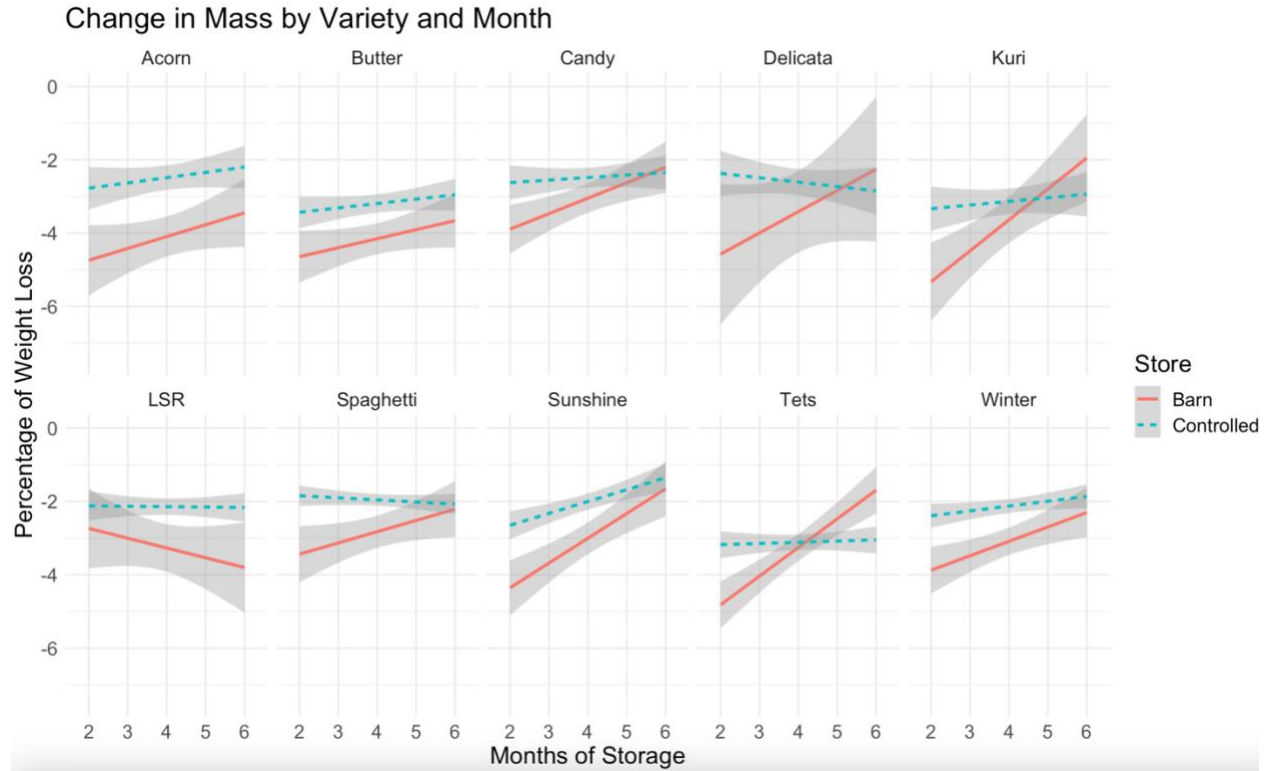


Figure 4: The percentage of Mass lost in each cultivar by month, examined in both storage environments. No change occurred during the first month of storage, so it is not recorded here. The shaded gray area represents a 95% C.I.

Amounts lost over six months in storage are detailed in table 9. In the analysis of mass, we saw a highly significant interaction ($P < 2.2 \times 10^{-16}$) between cultivar and storage of each variety. This was due to a size bias with our fruit going into storage from the beginning. There was no simple effect of storage on mass. The mass of each variety at the start of their storage periods varied, due to differences in size (figure 5).

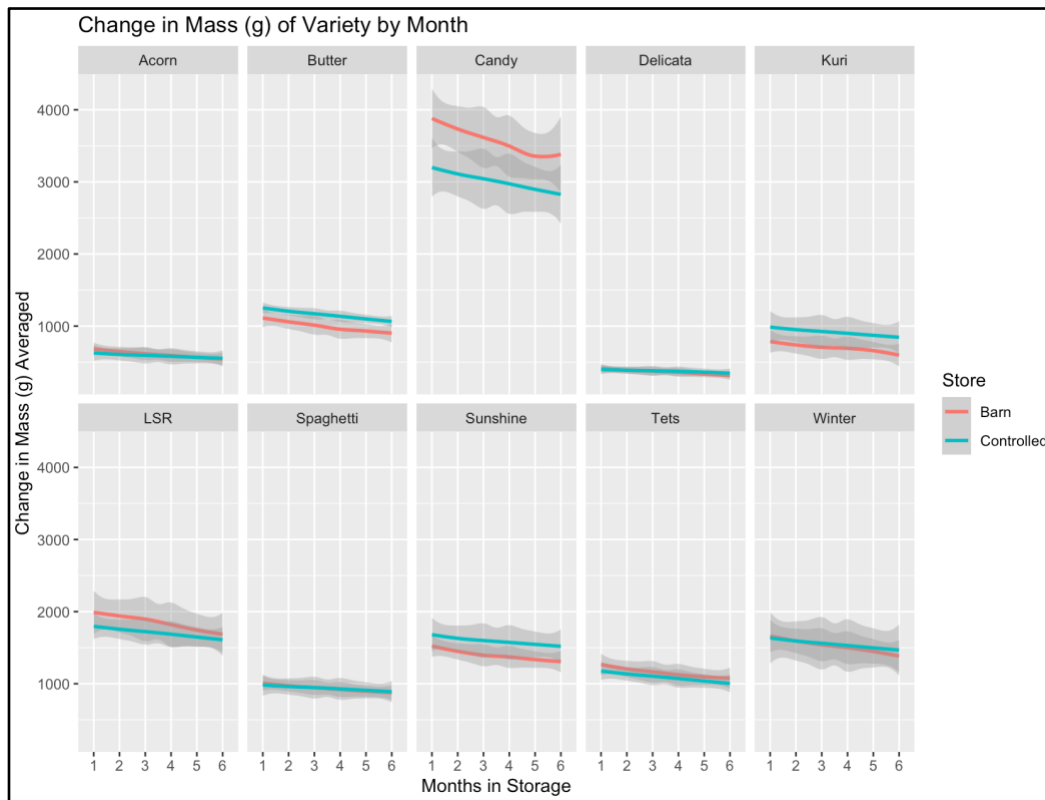


Figure 5: The change in Mass (g) of each cultivar examined in both storage environments.

The 'North Georgia Candy Roaster' started at an average weight of 3,500 grams, whereas the 'Honey Boat' starting weight averaged closer to 400 grams. All varieties examined, except for the 'Honey Boat', lost mass at a slower rate than the 'Starry Night PMR' variety did. (Figure 4).

Cultivar	Avg. % Change Barn	Avg. % Change Cooler	Difference
Winter Sweet	0.168	0.102	0.066
Tetsukabuto	0.153	0.146	0.006
Sunshine	0.142	0.096	0.046
Starry Night	0.208	0.118	0.090
Red Kuri	0.212	0.147	0.065
Pinnacle	0.133	0.094	0.039
NGCR	0.153	0.118	0.035
Metro PMR	0.191	0.150	0.041
LSR	0.148	0.102	0.046
Honey Boat	0.230	0.133	0.097

Table 9: Total percent of mass lost by variety, in each storage environment. All varieties of squash lose weight in storage. The variety of squash and storage environment affect how much weight is lost.

Color

There was also a highly significant effect of cultivar on the interior flesh color of each squash cultivar ($P < 2.2e-16$). Full CIELAB coordinates are not reported here, but rather the a^* score only. The a^* values of each cultivar increased with time ($P = 7.287e-07$) (Figure 6). This a^* value coordinate illustrates the increase in depth of color from a lighter pale yellow to deeper and darker orange or red. Color continues to develop after harvest when winter squash is placed in storage, and more rapidly at warmer temperatures (Beever et al. 1991, Wright and Grant, 1999).

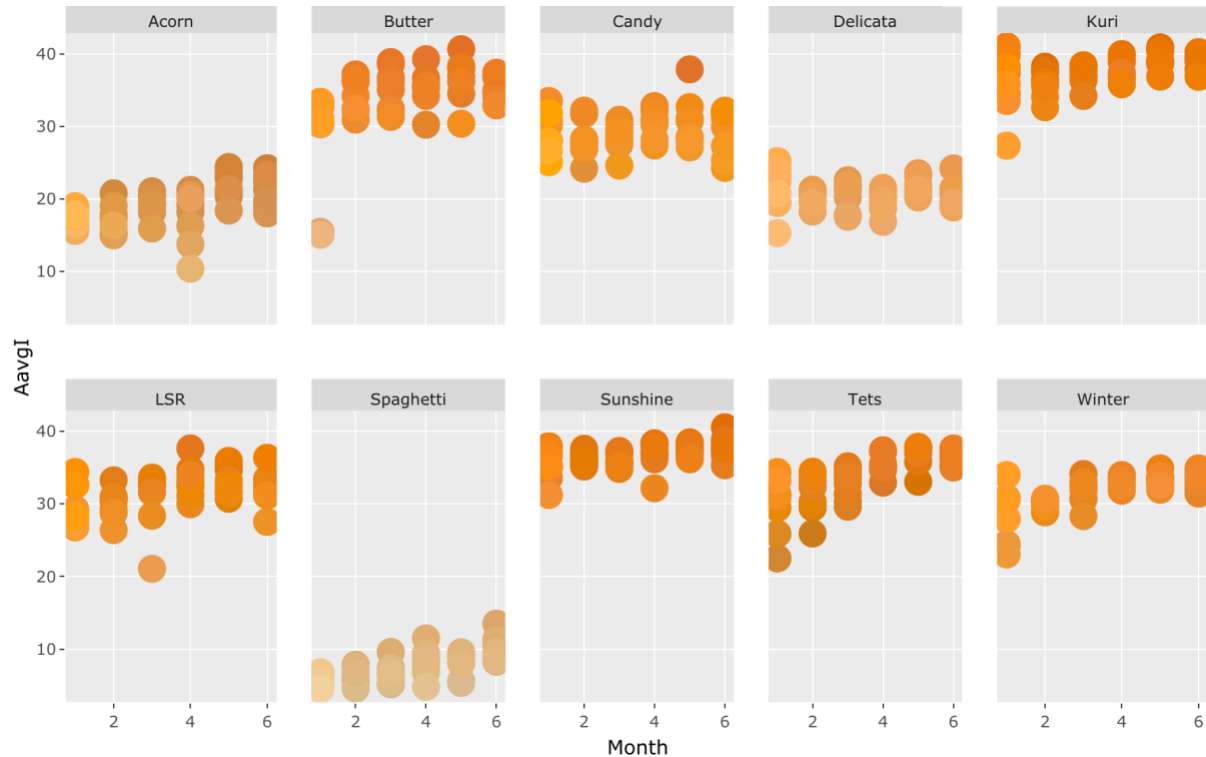


Figure 6: Change in a^* Value by Variety and Months in Storage. AavgI = a^* value, which illustrates the change in hue and depth of redness of flesh color in each variety over time, averaged over both storage environments. Butternut sees a more extreme magnitude in change of color when compared to Honey Boat or Sunshine.

Discussion

The goals of this project were to 1) determine how the flesh of 10 individual cultivars of winter squash responded to 2) six intervals of a monthly time period 3) in two unique storage areas. Winter squash are known as a storage vegetable, and research of this kind has taken place in other regions of the United States and around the world. However, we chose to replicate that research in Montana because our growing season is shorter, so winter vegetable storage is a necessity, and because our climate is much drier than the subhumid regions where previous research has taken place. The goal is to help local farmers and consumers understand which varieties they should choose for storage and how long to store them for, and when they should be

eaten. This will help producers market their winter squash more efficiently and increase the availability of local produce to consumers, which should increase the amount of winter squash being grown in Montana.

Cultivar

For every quality parameter that was measured, the magnitude of difference between cultivars was equal to or greater than any difference that occurred between time periods or storage environments. In short, it is the cultivar itself that will enable a winter squash's short or long storing ability. This is contrary to what Moon and X found in their 1963 research that analyzed how quality responded to storage periods in 36 varieties of winter squash. During the analysis of constituents, their research concluded that the age of the fruit, or amount of time that it was in storage, was a greater source of variation than cultivar.

The trends that we saw within SS, DM, starch, interior flesh color, and mass of each variety seemed to follow the general trends of previous research. Color, measured by a* value increased before decreasing again. Starch decreased while soluble solids increased. Dry matter gradually decreased, and mass was lost over time. However, in each of these quality parameters the degree and rate of change was unique to each variety.

Earlier classical papers (Merrow and Hopp, 1961; Phillips, 1946; Schales and Isenberg, 1963) often report butternut varieties as the best quality in comparison to others because of their high SSC and deep color. However, since the publication of those papers, there have been improvements to other winter squash varieties and so our results would suggest that other varieties have higher levels of soluble solids and color when compared to our measurements of the butternut variety we examined. The amount of soluble solids in 'Metro PMR' is nearly the

same as what Phillips had reported for their butternuts in 1941, however our kabocha varieties are much higher in soluble solids (10-15 g) than the buttercup varieties he reported on (8g) after three months of storage, and therefore would be considered sweeter and more appealing

Time Period

In our analysis of how time periods affect the quality of winter squash, we saw that time was equally significant to cultivar regarding color and starch content. The difference of means in mass, dry matter and soluble solids over time were significant but to a much lesser degree. So, while winter squash is being stored, the difference in means of color and starch content per cultivar are changing as time passes.

Phillips reported butternut as having a higher percentage dry weight of starch than buttercup over each stage of time in storage, though his starch trends also decreased as time passed. In contrast, we found that 'Winter Sweet', 'Sunshine', and 'Tetsukabuto' (comparable kabocha varieties from our experiment) had much higher levels of starch and DM at each level of time than the 'Metro PMR' variety of butternut we selected. At three months of storage the 'Winter Sweet' and 'Tetsukabuto' varieties had a higher level of starch than the butternut we chose did.

There are no other reports available that consider how starch or other quality parameters respond to time in storage for other varieties. Although acorn and spaghetti are well known types of winter squash, and 'Honey Boat', 'Tetsukabuto', and 'Red Kuri' are growing in popularity, the 'Lower Salmon River' and 'North Georgia Candy Roaster' varieties are open pollinated and considered heritage varieties that would not be well suited for consistent phenotyping. Acorn and spaghetti types seem to dominate the commercial market, which may be due to their earlier

ripening. Butternut is also a common type but is usually used for processing and not fresh eating. Since storage involves time and additional resources, farmers may be hesitant to hold on to squash for any longer than they need to. In New Zealand, time as an effect on *Cucurbita maxima* storage has been studied at shorter intervals and in regard to storage in cooled containers during shipment to Japan (Wright and Grant, 1999).

Storage Environment

Since a deeper orange flesh color is considered more appealing to consumers, it is beneficial to know how this sensory attribute changes while in storage. Interior flesh color was the only quality parameter where we saw an effect of the storage environment in our analysis, although it was much less significant than the effect of cultivar or time. The a value that was used as a way to compare the flesh color between the different cultivars we analyzed for this project measures how red any given squash is. The color red increases over time in all varieties and in both storage environments, however the amount of red, and the rate of change in color is unique to the variety itself. The 'Metro PMR', 'Sunshine', and 'Red Kuri' varieties start out much redder than the others, and 'Sunshine' and 'Red Kuri' see less of an increase in color than the 'Metro PMR' does. 'Starry Night' starts off redder than 'Pinnacle' but less red than 'Lower Salmon River', 'Tetsukabuto' and 'Winter Sweet'. 'Starry Night', 'Pinnacle', 'Lower Salmon River', 'Tetsukabuto' and 'Winter Sweet' all increase in redness by the same degree. Most varieties continue to gain color through the fifth month of storage. The 'North Georgia Candy Roaster' had a greater increase of color in the controlled cooler than in the heated barn, while in comparison the 'Tetsukabuto' became less red in the controlled cooler when compared to the heated barn. During the month of January, we saw a lower increase of the color red in the cooler

than in the barn. This may be explained by the barn's higher temperatures that month, which are known to cause a greater increase in color (Beever 1991, Wright & Grant 1999).

Conclusion

Local farmers growing winter squash in semiarid climates like Montana should prioritize choosing varieties that fit their market needs. Although storage requires protection from freezing temperatures, the safe use of a small heating unit inside of a storage facility should meet the needs of any farmer who is looking to increase their sales by storing winter squash through the winter months. In this study aimed at understanding storage in semiarid Montana, we found that the storage environment does not have a significant effect on any of the sensory attributes that are used to measure quality characteristics of flesh in winter squash.

Since it is the variety itself that enables long storing of winter squash, farmers should focus on becoming more familiar with varieties and their composition. When choosing what varieties of winter squash to grow and store, a farmer should be cognizant of the duration of time a variety can store well for. If the farmer wants to sell all their squash right after harvest, they should focus on selecting varieties that ripen sooner like acorn, spaghetti, and delicata types. If they would like to provide winter squash to consumers around the holiday season, then butternut and acorn types or varieties similar to 'Red Kuri' and 'Sunshine' would be a good fit. For farmers that would like to focus on late season winter market sales, choosing kabocha varieties with high dry matter and starch content would enable them to sell well into the winter months. Developing a marketing and sales plan according to the seller's ability and consumers desires would be a good choice for all involved. In smaller local markets farmers typically choose varieties for their eating quality, and which allows the consumer the benefit of knowing when the squash was

harvested and how long it has been stored for. In semiarid climates, if farmers are marketing their squash based on storage ability, then the risk of product loss is much less of a concern.

Future research may include repeating this experiment to see how results compare over additional years. It would be beneficial for farmers to know and see how a marketing plan that included the cost of storage and winter squash sales into March panned out. This may be possible through a SARE graduate student grant that included a partnership with local vegetable farmers. A more in-depth study that included a section on sensory evaluation through the storage periods would also be helpful, since during the later months of storage it appeared as though some squashes were dryer than when we had started.

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