Effects of Time, Temperature, and Cooking Vessel on Antioxidant Potential, PH, and Degrees Brix in the Processing of Black Garlic

Victoria Gargano

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ABSTRACT

Garlic, from the *allium* family, is known to contain organosulfur compounds. These compounds contain antioxidants, which provide a plethora of potential health benefits. Black garlic is created by processing regular garlic at temperatures ranging between 60-90°C, with high humidity for upwards of one month. The process of making black garlic alters these components, potentially increasing the antioxidant capacity. This end product yields a garlic that is a deep black color with a sweet flavor, pungent aroma, and a soft malleable texture. Black garlic is increasing popularity within the culinary world with more products making it to market shelves.

PURPOSE: To determine the effects that time, temperature, and cooking vessel has on the antioxidant potential, PH, and Brix in the production of black garlic. MATERIALS AND METHODS: Garlic was processed in a sous vide, fermentation station, and dehydrator at 57°C, 63°C, and 68°C for 25 days, 30 days, 35 days, and 40 days. The antioxidant potential was assessed using a Trolox Equivalency Antioxidant Capacity (TEAC) Assay for all 36 samples. Samples were diluted with distilled water and homogenized for PH and Brix readings. A PH meter was used for PH assessment, and a refractometer was used for Brix assessment. An ANOVA analysis was performed to assess the results. RESULTS: The TEAC values observed for the samples prepared by sous vide (21.82 uMol Trolox/g wet weight black garlic sample) were significantly higher than those prepared by dehydrator 12.35 82 uMol Trolox/g wet weight black garlic sample) and fermentation station (7.24 82 uMol Trolox/g wet weight black garlic sample), see Figure 1. Temperature was also significant on TEAC values (p = 0.004), samples prepared at 68°C (18.60 82 uMol Trolox/g wet weight black garlic sample) were significantly higher than those prepared at 57°C (9.56 82 uMol Trolox/g wet weight black garlic sample), and those prepared at 63°C (13.23 82 uMol Trolox/g wet weight black garlic sample) were not
significantly higher than those prepared at 57°C (9.56 82 uMol Trolox/g wet weight black garlic sample) (p= 0.325), and not statistically significant between those prepared at 63°C (13.23 82 uMol Trolox/g wet weight black garlic sample) and 68°C (18.60 82 uMol Trolox/g wet weight black garlic sample) (p= 0.096). There was no significant effect of time (p = 0.832), and no significance in any combination of time comparisons (all p values >0.05). There are also significant interaction effects between machine and time (p = 0.034). There is a positive correlation between temperature and TEAC value, showing that for every 1°C increase in temperature, a prediction of an increase of 0.812 TEAC value can be expected. The PH test resulted in a significant difference among temperature, time, and machine. For Temperature, the post-hoc tests show that of 57°C (5.01) was significantly higher than 68°C (4.74) (p < .001), 63°C (4.93) was significantly higher than 68°C (4.74) (p < .001), but not between 57°C (5.01) and 63°C (4.93) (p = .096). For time, 25 days (5.02) was significantly higher PH than 30 days (4.86) (p = .003), 25 days (5.02) was significantly higher than 35 days (4.87) (p = .006), 25 days (5.02) was significantly higher than 40 days (4.81) (p < .001). There was no significance found between 30 and 35 days (p = .995), or 30 and 40 days (p = .604), or between 35 and 40 days (p = .454). For machine, dehydrator and fermentation station, and sous vide (all p-values < .001). The fermentation station (5.15) showed significantly higher PH than both the dehydrator (4.87) and the sous vide (4.65). The dehydrator (4.87) showed significantly higher PH than then sous vide (4.65). It is predicted that for every 1°C increase in temperature, there will be a -0.024 decrease in PH, and for every 1 day increase in time, there will be a -0.013 decrease in PH. The Brix tests resulted in a significant difference among time, temperature, and machine. For temperature, the post-hoc tests show that 57°C (52.81) was significantly lower than and 68°C (56.875) (p = 0.050), but not between 57°C (52.81) and 63°C (54.79) (p = 0.467), 63°C (54.79) and 68°C
For time, the post-hoc tests show that 25 days (52.94) was significantly lower than 30 days (58.19) (p = 0.013) only, but not between 25 days (52.94) and 35 days (53.75) (p = 0.784), 25 days (52.94) and 40 days (55.42) (p = 0.287), 30 days (58.19) and 35 days (53.75) (p = 0.114), or 30 days (58.19) and 40 days (55.42) (p = 0.480), or between 35 days and 40 days (p = 0.822). For machine, the dehydrator (83.96) was significantly higher than the fermentation station (51.04) (p < .001), the fermentation station (51.04) was significantly higher than the sous vide (29.48) (p < .001), and dehydrator (83.96) was significantly higher than the sous vide (29.48) (p < .001). The linear regression model for Brix had an R-squared value of 0.000, indicating that the model did not explain any of the variance in the data. CONCLUSION: Machine and temperature had the most significant effects on the TEAC values, though the fermentation station and dehydrator were not significantly different from one another’s results. Time did not have a significant effect on TEAC values. Results predict that for every degree Celsius the temperature is increased, there will be a 0.812 increase in antioxidants. The test results indicate that the effects time, temperature, and machine have on PH are all significant variables. The results also indicate a correlation between temperature and PH, as well as time and temperature on PH. The Brix results indicate that time, temperature and machine all had a significant effect on the degrees Brix. However, there was no definitive correlation found between the variables.
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Effects of Time, Temperature, and Cooking Vessel on Antioxidant Potential, PH, and Degrees Brix in the Processing of Black Garlic

By:
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CHAPTER 1: INTRODUCTION

1.1 INTRODUCTION TO BLACK GARLIC

Black garlic is the result of processing normal garlic under heat and moisture for extensive periods of time. The process varies slightly depending on the producer, and will be discussed in more detail later on. The end results of creating black garlic yields a garlic that is a deep black color, sweet flavor, pungent aroma, and has a soft, malleable texture (Shunsuke Kimura; Yen-Chen Tung; Min-Hsiung Pan; Nan-Wei Su; Ying-Jang Lai; Kuan-Chen Cheng, 2017). This product has had many uses and claims made towards it, some of which are unclear.

Black garlic, commonly referred to as fermented garlic, is an ancient ingredient originating somewhere in Asia, with several health claims behind it. Though the exact origin is unknown, for centuries it has been a prominent part of Korean, Japanese, and Thai cuisines (Katarzyna Najman; Anna Sadowska; Ewelina Ballmann, 2020). A controversial aspect of black garlic is whether or not it is truly a fermented product (Weissman, 2019). The normal processing temperature of black garlic is high for a ferment, but low for cooking. There are several reactions, such as the Maillard reaction, that occur, but only one recent study mentions the bacterial components present in black garlic (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018). It seems that this study is not widely known, and the chefs of the culinary world still argue about the truth of this (Weissman, 2019).

The goals of this research are to see which processing machine, duration of time, and temperature used in processing black garlic will result in the highest antioxidant activity. These variables were used to evaluate the antioxidant effects that the process has on the garlic. The PH and degrees Brix will also be calculated in this experiment in order to better understand the
variables’ effects on the product. This will help create a foundation for further future research, and even production of black garlic. The PH will help determine food safety characteristics, while the degrees Brix can potentially aid in predicting the desired sensory characteristics for producers in the food industry.

1.2 HISTORY OF GARLIC

While the origins of black garlic are in question, the use and utilization of garlic can be seen across the globe, in many different cultures. Evidence of humans eating garlic goes all the way back to over 10,000 years ago. Garlic is thought to be one of the first domesticated crops early humans cultivated when transitioning from a nomadic lifestyle to civilized living. Through the years, garlic was commonly used for travel on long journeys due to its light weight, nutrient dense, and extended shelf-life capacities. Its sharp aroma and pungent flavor was also good for masking the flavor of rotting food (Cherry, 2014).

Throughout history, the influence of garlic on culture is evident. In ancient Egypt, garlic was fed to slaves to keep them strong and able to build the pyramids. When the garlic rations were reduced, the slaves revolted and started a strike of sorts, all due to garlic. In ancient Greece, Olympians would eat garlic as a performance enhancing drug to build strength and keep their stamina high. Roman warriors would carry and eat garlic in battle, and when the battle was over, they would plant garlic in the fields, spreading it along their path (Cherry, 2014).

The Silk Road brought items in all directions throughout the Asian and European continents. Garlic was among the travelers and helped shaped cultures and cuisines as we currently know them. In China, the symbol for garlic is made up of a single character, showing that it most likely originated from the first version of their language. The various regional cuisines of China vary
greatly, but all include the influence of garlic. Festivities and celebrations include the utilization of garlic in their culture (Cherry, 2014).

Many cuisines include notable influence of garlic, including the base to many different cultural cuisines. In the culinary world there is a French term, mirepoix, which is used to explain the “holy trinity”, which provides the base ingredients to almost all their dishes. In France a mirepoix consists of carrots, celery, and onions (America, 2006). In many other cultures, their trinity contains garlic among their ingredients. India’s version of a mirepoix is ginger, garlic, and onions. While China’s version is ginger, garlic, and scallions. These are used as a base to most of their culturally traditional dishes, showing just how deeply regarded garlic is throughout the world (Cherry, 2014).

1.3 ANCIENT HEALTH BENEFITS AND BELIEFS OF GARLIC

The first known mention of garlic being utilized for medicinal purposes comes from an Ancient Egyptian papyrus. It was discovered by a German archeologist in 1872. The perfectly preserved papyrus is estimated to date back to 1552 BC, and is thought to be copied from even earlier texts (Cherry, 2014).

Greek mythology has a story about Apollo’s son, Asclepius, who practiced the art of healing herbs. It was said that his healing advancements made him capable of raising the dead. This made Hades and Zeus uncomfortable, especially because he was tampering with the natural order of life. While writing his formula for immortality down, Zeus struck down Asclepius. Zeus saw what he was recording, and sent down a rain to destroy his work, washing it into the ground. When the sun rose, plants sprang up from where the formula was washed away. Those plants were garlic, indicating that it was believed to be a part of the immortality recipe. Asclepius had many children, some who followed in his footsteps as healers. He had two daughters named
Hygieia and Panacea. Hygieia is the goddess of health, and is where the word hygiene derived from, and Panacea is the goddess of universal remedies, and where the term panacea comes from, meaning “cure-all”. (Cherry, 2014). This story helps paint a picture of the influence garlic had on early medicine.

In ancient Rome, the philosophers Hippocrates and Dioscorides both used garlic in their healing methods. Hippocrates is noted as the father of modern medicine, and founded the first school of medicine. Dioscorides is noted as the father of modern pharmacology, and wrote one of the most valued books on herbal healing. In their time, both had prescribed garlic to patients for various ailments including pulmonary disease, tumors, and for cleansing the arteries (Cherry, 2014).

Following the time of Dioscorides, Pliny the Elder wrote an entire medical encyclopedia. In this encyclopedia he recommends garlic for over sixty ailments ranging from the protection from infections and toxins to tuberculosis. After him came a Roman physician and philosopher named Galen. He treated gladiators’ wounds with bandages that were soaked in garlic juice, and held the claim to never having lost a patient (Cherry, 2014).

The spice route was responsible for spreading garlic through most Asian regions. This was one of the catalysts to the beginning of traditional Chinese medicine. Traditional Chinese medicine promoted the use of garlic to aid in digestion, respiratory issues, diarrhea, worms, depression, fatigue, headaches, and insomnia. At that time cancer was described as stagnation and the coldness of the body, and in traditional Chinese medicine they believed the “warmth” that garlic provided would help combat it (Cherry, 2014).
From China garlic moved to Korea, where it was considered one of their first medicines. It is even in the folklore of their creation. From Korea garlic made way to Japan. Japan uses garlic in their Kampo teachings of botanical medicine (Cherry, 2014).

Garlic migrated into India where it was utilized and adapted in many ways. The Bower Manuscript calls garlic “the universal remedy”. It was commonly prescribed to treat infections, parasites, weakness and fatigue, as well as a variety of digestive issues. Charaka Samhita is considered the father of traditional Indian medicine. He commonly recommended garlic as a diuretic, heart stimulant, and digestive aid. He also utilized it as treatments for heart disease, eye ailments, and arthritis. Garlic was believed to be a cure for cholera, colic, dysentery, hardening of the arteries, tuberculosis, and typhoid (Cherry, 2014).

It is obvious that the historical influences of garlic through the ages have shown the promise of medicinal properties. Modern medicine has proven and showed that there are many benefits and possible truths behind the mythologies and ancient remedies of the past.

1.4 POPULARITY INCREASE AND REACTIONS
Pittsburgh Tribune launched an article about black garlic, and the popularity it was gaining back in 2009. Black garlic was patented by Scott Kim from South Korea in 2004, though it is known to have been around for hundreds of years prior to that. He planned on marketing the black garlic as a super food, and it took off in the culinary world due to its unique flavor (Kim, 2009). Chefs, such as Joshua Weissman, claim that the process of making black garlic is actually not considered a ferment, and only holds the presence of the Maillard reaction, which is why the garlic turns its signature black color. Though many processes happen in the formation of black garlic, the Maillard reaction is clearly not the only chemical reaction that could be going on (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao,
2018). This confusion is something that needs clarification in order to provide the appropriate classification of what black garlic truly is to the culinary world. (Weissman, 2019).

1.5 BIOLOGICAL AND CHEMICAL COMPOSITION

The same chefs that claim fermentation is not going on within the process of making black garlic, seem of focus only on the presence of caramelization and the Maillard reaction (Weissman, 2019). Both reactions are notable and present in black garlic. Harold McGee describes caramelization as a complex series of reactions that involve the deepening and enrichment of sugars. This reaction uses sugar as a substrate, and tends to promote the formation of naturally occurring acidic compounds. The results are usually a sweeter tasting product with a darker color (McGee, 2004).

Though caramelization is responsible for some of the color change in black garlic processing, it is not the only color altering reaction. The Maillard reaction is another common reaction that occurs in making black garlic, though it provides more of black garlic’s signature black color than caramelization. This reaction’s substrates are both carbohydrates and amino acids that aid in developing flavor of color of the black garlic (McGee, 2004).

Unlike salt fermented items, acid fermented vegetables reduce the growth of undesirable microorganisms by promoting good bacteria to produce high levels carbon dioxide and acid, which will rapidly lower the PH, killing off the bacteria that cannot tolerate the harsh environment. The bacteria that are naturally found in vegetables and could survive this type of environment are related to lactobacillus, and can even survive human digestive systems. These lactobacillus related bacteria found in acidic fermented vegetables also play a role in digesting and converting sugars in this process, which is the reason the degrees Brix was calculated in this experiment (Campbell-Platt, 2018). Another way of recognizing whether or not an item is
fermented is by monitoring the PH levels throughout the process. If the PH lowers, then that indicates that an acid fermentation is occurring. Acid fermented vegetables are high in vitamins and minerals (Campbell-Platt, 2018).

Though there is controversy involving the status of black garlic being a fermented product, there is one article that was found to include and involve the microbial breakdown of black garlic, proving that it is a ferment. This research performed involved the characterization of the microbial community that developed during the black garlic processing. The importance of this research is not only within the classification of black garlic as a ferment, but the mapping of these microbes can indicate the metabolic potential available in black garlic (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018).

The study showed evidence of bacterial growth of various categories. The bacteria that survived the process were those that customarily survive acidic fermentation processes. The microbiome functional analysis of this study showed evidence of increased membrane transport, amino acid metabolism, and carbohydrate metabolism. This evidence indicates that these are the key microbial metabolic processes that are taking place during black garlic production (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018).

The chemical components found in black garlic are dependent on the thermal processing procedure used to produce it (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020). Certain sugars and amino acids required for the Maillard reaction, but at high temperatures the rate of sugar reduction exceeds the reaction’s consumption rate. Though the higher temperatures reduce the processing time, it may compromise the sensory affects and flavor as described in this study (Shunsuke Kimura; Yen-Chen Tung; Min-Hsiung Pan; Nan-Wei Su; Ying-Jang Lai; Kuan-Chen Cheng, 2017). The roll sugar plays in formation of black garlic opens up the idea that
lower temperatures for longer duration of time may be able to reduce the sugar loss and increase
its presence in reaction consumption instead. Theoretically, the lower temperatures may yield a
superior product (Shunsuke Kimura; Yen-Chen Tung; Min-Hsiung Pan; Nan-Wei Su; Ying-Jang
Lai; Kuan-Chen Cheng, 2017).

In a study reviewing the chemical and biological properties of aged garlic extract, the
authors compare black garlic to fresh garlic bioavailability of specific compounds in both rats
and dogs, *in vitro* and *in vivo*. The biological availability of a sulfur-containing amino acid S-1-
propenyl-L-cystein (S1PC) and its beneficial effects were analyzed in this study. This compound
is found in minimal amounts in fresh garlic, but greatly increases during the black garlic aging
process. This systematic review explains several chemical changes and isomerization that occur
during the production of black garlic. It shows the changes and morphing of various organosulfur
components that help create black garlic and make it as beneficial as the claims state
(YUKIHIRO KODERA, MASADIO KURITA, MASATO NAKAMOTO and TOSHIAKI
MATSUTOMO, 2020) (Yukihiro Kodera, Mitsuyasu Ushijima, Hirotaka Amano, Jun-ichiro

One study highlights the component in garlic that is related to the plant’s defense
mechanism, allicin (Rahman, 2007). Allicin is produced by garlic in an enzymatic reaction when
it is injured. This component protects the plants from infestation of fungi and insects. It is
referred to as mother nature’s insecticide. The structure of allicin changes during the black garlic
processing. This structural change is responsible for making the allicin within the garlic more
stable and biologically available. The more biologically available the allicin is, the more a
person’s body will be able to absorb. Thus, increasing the availability of the health benefits
associated with allicin. These benefits include antimicrobial function, anti-cancer, reducing the
risk of cardiovascular disease, improving immune function, and even having effect on proteins and lipids within the body (Rahman, 2007).

In a study conducted in Poland, researchers discuss the bioactivity, antioxidant, and physiochemical properties of black garlic being produced with conventionally farmed and organically farmed garlic. All garlic used in the experiment was processed at 70°C with 80% humidity for 45 days. The study also reveals that the usual and more common processing procedures used for making black garlic are temperatures that generally range from 60-90°C, with the humidity ranging from 70-90%, and the processing time being anywhere in the range of 30-90 days. This information is taken into consideration when creating the methodology for the experiment. The study concluded that there is a significant increase in the phenolic compounds when garlic is processed into black garlic, and there was even a significantly higher level of antioxidants in the organically grown garlic compared to the conventionally grown garlic that was processed (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020). This information can be used for future research, along with the results of this experiment.

Another similar study was performed using various species of garlic in Thailand. This experiment focused on the process of making black garlic using different varietals of garlic to see if they yielded significantly different physiochemical properties. The results of this study showed that certain varietals of garlic, when processed into black garlic, did in fact produce significantly different physiochemical properties, including phenolic compounds. The study used 6 species of garlic that are commonly found in Thailand, with 3 of the samples showing higher results than the rest. All of the garlic underwent the same processing procedure which was 75°C, and 90% relative humidity, for 15 days (Piyachat Sunanta; Hsiao-Hang Chung; Kaewaling
This is a much shorter time than most procedures seen in other studies. The majority of the research that is out on black garlic is, understandably, about utilizing various pretreatments to reduce the time in which it takes to make black garlic. The time component of producing black garlic is one of the factors that deters chefs and home cooks from producing it themselves. However, this experiment utilizes time as one of the manipulated variables in order to calculate the effect it has on the ending black garlic product.

1.6 HEALTH BENEFITS

Modern medical research shows that there are many true medicinal properties found in garlic. Garlic is known to have natural antibiotic properties that can aid in the prevention and reducing discomfort from infections such as yeast infections, diaper rash, and even fungal infections such as ringworm, jock itch, and even athletes’ foot. Raw garlic can help rid the body of intestinal parasites. Garlic has shown effectiveness in killing viruses such as viral meningitis, viral pneumonia, influenza and even herpes (Cherry, 2014).

Today garlic is used in supplements to help support the maintenance of healthy cholesterol levels, blood circulation, and immune and liver function. Studies have shown that garlic can help lower blood pressure and slow down the progression of atherosclerosis. Research shows that garlic helps blood circulation by aiding blood’s production of hydrogen sulfide. Decreased levels of hydrogen sulfide have been linked to Alzheimer’s and Parkinson’s disease. Garlic also acts as a blood thinner, which can help prevent heart attacks and stroke. Garlic has been linked to boosting immune function and stimulating the activity of natural killer cells, t-helper cells, and macrophages (Cherry, 2014).
Garlic is naturally high in antioxidants that come from its organosulfur compounds. These compounds are shown to aid in cellular repair, and the breakdown of toxins. It contains glutathione which is found to be deficient in those who suffer from heart disease, liver disease, cancer, diabetes, Alzheimer’s, Parkinson’s, asthma, and arthritis (Paola Tedeschi; Manuela Nigro; Alessia Travagli; Martina Cantani; Stefania Merighi; Stephania Gessi, 2022). Garlic may help lower the risk of pre-eclampsia in pregnant women. It has shown potential to reduce the risk of cancer, especially cancer relating to the intestinal track. It is shown to have anti-inflammatory properties, and even aid in the detoxification of heavy metals in the body (Cherry, 2014).

Garlic is known for a plethora of potential health benefits, but there are many different types of garlic and ways to consume it. There are a multitude of studies that test the effects garlic has on various illnesses, while little is specifically done on black garlic. Therefore, the majority of the following studies mentioned include the benefits of garlic in general. Black garlic begins as regular garlic, and a few studies show that the process may increase the bioavailability of the various garlic components, which could theoretically increase the beneficial claims of normal garlic (Rahman, 2007) (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020) (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018) (Shunsuke Kimura; Yen-Chen Tung; Min-Hsiung Pan; Nan-Wei Su; Ying-Jang Lai; Kuan-Chen Cheng, 2017).

One study focused on garlic and anti-cancer fighting properties of various different ways to consume it, such as: supplements, raw, cooked, and aged (black garlic). Though the authors concluded that the results were inconsistent for all cancers, they also stated that the complexity of garlic in its various forms may be the reason behind the inconsistent findings, though there are some studies that suggest that aged black garlic has anticarcinogenic properties that aid in cancer
fighting and prevention (Zeinab Farhat; Pamela A. Hershberger; Jo L. Freudenheim; Manoj J. Mammen; Rachael Hagerman Blair; Diana S. Aga; Lina Mu, 2020). Another study focused on the effect black garlic has on lipid metabolism. The study concluded that aged black garlic at certain concentrations may aid in the promotion of lipolysis, the catabolic breakdown of triglycerides, while also inhibiting lipogenesis, the anabolic storage of fat in adipose tissue within the body. There is no doubt that garlic has a multitude of potential health benefits, but very minimal research is done on the production methods and figuring out which of the processes will yield the most beneficial version of black garlic (Hajin Nam; Harry Jung; Yooyeon Kim; Boyoung Kim; Kyeong Hee Kim; Sang Jae Park; Jun Gyo Suh, 2018).

Garlic has demonstrated its ability to provide health benefits. It has been used as medicine for centuries, and is one of the earliest documented medicinal plants. It has been used to treat various diseases including leprosy, ear diseases, parasitic infections, abdominal pain, and infections (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020). Black garlic has showed signs of possibly increasing these properties in garlic, and making them more biologically available (Rahman, 2007).

Black garlic has been related to aiding in the prevention of cardiovascular disease, supporting blood vessel health, lowering blood pressure, and even lowering low density lipoproteins (LDL), or bad cholesterol, while raising high density lipoproteins, or good cholesterol (Hajin Nam; Harry Jung; Yooyeon Kim; Boyoung Kim; Kyeong Hee Kim; Sang Jae Park; Jun Gyo Suh, 2018). Black garlic also contains organosulfur components. These components found in black garlic are water soluble and help fortify liver production. It aids in the promotion of removing toxins from the body as well as helps prevent damage to liver cells caused by over-the-counter pain relievers (Gehan Moustafa Badr; Jawaher Abdulaziz AL-Mulhim, 2014). These
organosulfur components also promote enzymatic reactions that occur in the liver. Black garlic has been shown to boost immune system killer cells, aiding in fighting bacteria and viruses. It has even shown the potential to slow the body’s histamine response, which is responsible for the body’s reaction to allergens (Benson, 2015).

Aged black garlic was studied in rats for its benefits regarding cerebral damage and inflammatory response after ischemia. The study concluded that the black garlic had more than just antioxidant related beneficial effects on the brain. The aged garlic extract treatment diminished neurological alterations, as well as histological damages induced by cerebral ischemia. The treatment also proved to reduce the effect of post-injury related enzymes and proteins, and showed signs of aiding in inflammation (Ana Laura Colin-Gonzalez; Alma Ortiz-Plata; Juana Villeda-Hernandez; Diana Barrera; Eduardo Molina-Jijon; Jose Pedraza-Chaverri; Perla D. Moldonado, 2011).

Along the same line of researching neurological benefits of black garlic components, another study focused on it effects against Alzheimer’s Disease. Alzheimer’s Disease, and other neurological degenerative diseases, are plaguing an aging society. Allicin, and other garlic components, have been found to help prevent some of the neurological effects of this disease (Paola Tedeschi; Manuela Nigro; Alessia Travagli; Martina Cantani; Stefania Merighi; Stephanie Gessi, 2022).

The effects of black garlic and its potential for medical benefits is commonly researched. There are studies that showcase the effects of garlic and its various components in regards to fighting cancer. In one study, the authors focused on the various ways to ingest and consume garlic and the potential benefits and components each particular preparation contributes to the consumer. There are various components that are prevalent in garlic that have been proven to aid
in the processes involved in the battle of cancer. Garlic extracts have been shown to increase enzyme activity and inhibit the cancerous endothelial cells in mice. There is evidence that shows that garlic aid in reducing tumor growth in patients in regards to certain types of cancers such as skin and colon cancer. The antioxidants present in black garlic have been shown to prevent oxidative stress damage that can be caused by cancer cells. The various components found in garlic are discussed in regards to solubility and efficacy of consumption. There are beneficial components found in the various garlic products that are either water or fat soluble. This information helps relate specific information to bioavailability and utilizable components and benefits that are found in the different forms of garlic. (Zeinab Farhat; Pamela A. Hershberger; Jo L. Freudenheim; Manoj J. Mammen; Rachael Hagerman Blair; Diana S. Aga; Lina Mu, 2020).

Black garlic has been shown to provide a plethora of health benefits. There is hope that the research involved in this study will be beneficial in the advancement of some of this research. It would be interesting to see the effects from these studies if performed again from whichever sample yield the highest antioxidant potential. Comparing the differences will also help to determine a bottom line, or basis for production methods used to make black garlic. Having a generalized procedure that is proven to provide more beneficial results is important to use as a point of reference for additional research that can be performed. There is the potential that this information may also aid in the number of antioxidant activity that are utilized in supplement production.

Many people suffer from inflammatory issues regarding a variety of chronic diseases. Some people who struggle with these diseases may be interested in producing garlic at home to help with some of their chronic ailments and symptoms. This research will hopefully be able to help
reduce any trial and error for those seeking food as medicine, and any alternative or additional supplementation into their diets that can help them. The multitude of diseases or ailments that black garlic has shown to positively affect ranges from inflammation in the gut, cancer, neurological diseases, to even gingivitis (Paola Tedeschi; Manuela Nigro; Alessia Travagli; Martina Cantani; Stefania Merighi; Stephania Gessi, 2022) (Masahiro Ohtani; Tsubasa Nishimura, 2019) (Gehan Moustafa Badr; Jawaher Abdulaziz AL-Mulhim, 2014).

1.7 RESEARCH QUESTION
The main question of this research is what temperature, duration of time, and piece of machinery used to create black garlic will yield the highest number of antioxidants to achieve the optimal production method. The other questions to be answered in this study are the effects that temperature, time, and machinery have on the PH and degrees Brix during the black garlic processing.

1.8 IMPLICATIONS AND PURPOSE
The results from this experiment can help those at home as well as producers, doctors, patients, chefs, athletes and pharmaceutical companies to create a better product. The versatility and expansive benefits that this product encompasses can result in multifaceted uses and implications. The following paragraphs explain how this research can potentially help just a few of the industries and businesses with the knowledge obtained from the end results. These are all either currently used methods, or theoretical implication and application of black garlic.

Pharmaceutical companies use aged black garlic extract in medications and supplements. This information could change the chosen production methods and provide them with more superior products that may yield more promising results. This could help expand their outreach and marketing platforms as well. There is even research that combining black garlic with
methotrexate can create a more promising results due to the black garlic inhibiting the intestinal damage that methotrexate can cause in cancer patients (Tanvir Ahmed; ChinKun Wang, 2021). The antioxidant properties of the black garlic can be made in the most effective way, maximizing health benefits compared to products their competing companies are producing.

Doctors can recommend the preferred production method from this research to their patients. Those who experience inflammation and illnesses of various types may want to add black garlic into their diets as a supplement to aid in alleviating those symptoms. For example, those with Rheumatoid Arthritis may experience some pain relief from the symptoms that their disease causes (Cherry, 2014). The same concept can be applied to almost any types of arthritic and inflammatory diseases. The information provided in this research can help those interested in adding it to their diets, and willing to take on the time-consuming project, without having them wonder if the production method works. They will be able to begin the project with the knowledge of the best home production method that will produce the highest number of antioxidants. This will allow them to introduce the most effective way to achieve the most beneficial and bioavailable form of black garlic into their system.

Runners and other athletes may also benefit from adding this to their diet. Athletes tend to experience various amounts of inflammation due to the physical strain their training puts on their bodies. The soreness and aching inflammation that follows work outs and training can possibly be reduced by adding black garlic into their diet. This beneficial implication can be deduced from the affects that black garlic has on the inflammatory response produced by the immune system (Rahman, 2007).

The acidic fermentation, as well as the caramelization and Maillard reactions that take place in the processing of black garlic, are part of what affects the overall taste and texture of black
garlic (McGee, 2004). These are sensory attributes that can be measured in order to determine
the perception consumers have on products. The reason to measure the PH and degrees Brix is to
calculate the potential effects that time, temperature, and machine have on them. The information
provided can be utilized by food companies in order to determine how these variables are
affecting their product, and lead them in the right direction of a production method that will yield
a more desirable and consumer acceptable product (Campbell-Platt, 2018).

Companies producing black garlic may choose to utilize the results of this research when
developing their production procedures for making black garlic. They can possibly use this
information and provide transparency about their chosen production method to consumers. The
references in this article can be used to help expand target markets.

There are some culinary related black garlic items on the market. However, they are very
expensive and can substantially increase a restaurant’s food cost. There is also little information
about the process used by these companies, which may in fact involve a step that could
potentially reduce the health benefits and antioxidant capacity in the black garlic they are
producing (In Guk Hwang; Young Jee Shin; Seongeung Lee; Junsoo Lee; Seon Mi Yoo, 2012).
High end chefs also tend to prefer to make their elite culinary items in-house to ensure they are
made properly and to their standards. This information will allow them to do that, along with
complete control over their end product and utilization. The marketing aspects of making this
product in-house are also extremely beneficial for the business itself. The PH and Brix
assessments that were conducted can also aid chefs and producers in altering the process in order
to achieve their desired sensory characteristics in their end product.

As a former chef, this author has vast experience and culinary knowledge of the implications
of this research in the restaurant industry. This research will provide the knowledge of how to
create the most beneficial black garlic product using equipment that most industrial kitchens or homes, either already have, or can be easily obtained. The knowledge from this research will provide the appropriate preparation process, which in turn will save chefs and home cooks time and money if they desire to utilize this product.
CHAPTER 2: MANUSCRIPT I
TEAC Antioxidant Analysis of Black Garlic Production Methods
2.1 ABSTRACT

Garlic, from the *allium* family, is known to contain organosulfur compounds. These compounds contain antioxidants, which provide a plethora of potential health benefits. Black garlic is created by processing regular garlic at temperatures ranging between 60-90°C, with high humidity for upwards of one month. The process of making black garlic alters these components, potentially increasing the antioxidant potential. PURPOSE: To determine the effects that time, temperature, and cooking vessel has on the antioxidant potential of producing black garlic. MATERIALS AND METHODS: Garlic was processed in a dehydrator, fermentation station, and sous vide at 57°C, 63°C, and 68°C for 25 days, 30 days, 35 days, and 40 days. The antioxidant potential was assessed using a Trolox Equivalency Antioxidant Capacity (TEAC) Assay for all 36 samples. An ANOVA analysis was performed to assess the results. RESULTS: The TEAC values observed for the samples prepared by sous vide (21.82 uMol Trolox/g wet weight black garlic sample) were significantly higher than those prepared by dehydrator (12.35 82 uMol Trolox/g wet weight black garlic sample) and fermentation station (7.24 82 uMol Trolox/g wet weight black garlic sample), see Figure 1. Temperature was also significant on TEAC values (p = 0.004), samples prepared at 68°C (18.60 82 uMol Trolox/g wet weight black garlic sample) were significantly higher than those prepared at 57°C (9.56 82 uMol Trolox/g wet weight black garlic sample), and those prepared at 63°C (13.23 82 uMol Trolox/g wet weight black garlic sample) were not significantly higher than those prepared at 57°C (9.56 82 uMol Trolox/g wet weight black garlic sample) (p= 0.325), and not statistically significant between those prepared at 63°C (13.23 82 uMol Trolox/g wet weight black garlic sample) and 68°C (18.60 82 uMol Trolox/g wet weight black garlic sample) (p= 0.096). There was no significant effect of time (p = 0.832), and no significance in any combination of time
comparisons (all $p$ values $>0.05$). There are also significant interaction effects between machine and time ($p = 0.034$). There is a positive correlation between temperature and TEAC value, showing that for every $1^\circ$C increase in temperature, a prediction of an increase of $0.812$ TEAC value can be expected. CONCLUSION: Machine and temperature demonstrated significant effects on the TEAC values, though the fermentation station and dehydrator were not significantly different from one another’s results. Time did not have a significant effect on TEAC values. Results predict that, within the measured range of temperatures, for every degree Celsius the temperature is increased, there will be a $0.812$ increase in antioxidants.
2.2 INTRODUCTION

ANTIOXIDANT INTRODUCTION

Modern medical research shows that there are many true medicinal properties found in garlic. Garlic is naturally high in antioxidants that come from its organosulfur compounds. These compounds are shown to aid in cellular repair, and the breakdown of toxins. It contains glutathione which is found to be deficient in those who suffer from heart disease, liver disease, cancer, diabetes, Alzheimer’s, Parkinson’s, asthma, and arthritis. Garlic may help lower the risk of pre-eclampsia in pregnant women. It has shown potential to reduce the risk of cancer, especially cancer relating to the intestinal track. It is shown to have anti-inflammatory properties, and even aid in the detoxification of heavy metals in the body (Cherry, 2014) (Benson, 2015).

Garlic is known to have natural antibiotic properties that can aid in the prevention and reducing discomfort from infections such as yeast infections, diaper rash, fungal infections such as ringworm, jock itch, and even athlete’s foot. Raw garlic can help rid the body of intestinal parasites. Garlic has shown effectiveness in killing viruses such as viral meningitis, viral pneumonia, influenza and even herpes (Cherry, 2014) (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020).

Today garlic is used in supplements to help support the maintenance of healthy cholesterol levels, blood circulation, and immune and liver function. Studies have shown that garlic can help lower blood pressure and slow down the progression of atherosclerosis. Research shows that garlic helps blood circulation by aiding bloods’ production of hydrogen sulfide. Decreased levels of hydrogen sulfide have been linked to Alzheimer’s and Parkinson’s disease. Garlic also acts as a blood thinner, which can help prevent heart attacks and stroke. Garlic has been linked to boosting immune function and stimulating the activity of natural killer cells, T-helper cells, and macrophages (Cherry, 2014).
Garlic is known for a plethora of potential health benefits, but there are many different types of garlic and ways to consume it. There are a multitude of studies that test the effects garlic has on various illnesses, while little is specifically done on black garlic. Therefore, the majority of the following studies mentioned include the benefits of garlic in general. Black garlic begins as regular garlic, and a few studies show that the process may increase the bioavailability of the various garlic components, which could theoretically increase the beneficial claims of normal garlic (Rahman, 2007) (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020) (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018) (Shunsuke Kimura; Yen-Chen Tung; Min-Hsiung Pan; Nan-Wei Su; Ying-Jang Lai; Kuan-Chen Cheng, 2017).

Garlic has had many proven claims towards health benefits. It has been used as medicine for centuries, and is one of the earliest documented medicinal plants. It has been used to treat various diseases including leprosy, ear diseases, parasitic infections, abdominal pain, and infections (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020). Black garlic has showed signs of possibly increasing these properties in garlic, and making them more biologically available (Rahman, 2007).

Black garlic has been related to aiding in the prevention of cardiovascular disease, supporting blood vessel health, lowering blood pressure, and even lowering low density lipoproteins (LDL), or bad cholesterol, while raising high density lipoproteins, or good cholesterol (Hajin Nam; Harry Jung; Yooyeon Kim; Boyoung Kim; Kyeong Hee Kim; Sang Jae Park; Jun Gyo Suh, 2018). Black garlic also contains organosulfur components. These components found in black garlic are water soluble and help fortify liver production. It aids in the promotion of removing
toxins from the body as well as helps prevent damage to liver cells caused by over-the-counter pain relievers (Gehan Moustafa Badr; Jawaher Abdulaziz AL-Mulhim, 2014). These organosulfur components also promote enzymatic reactions that occur in the liver. Black garlic has been shown to boost immune system killer cells, aiding in fighting bacteria and viruses. It has even shown the potential to slow the body’s histamine response, which is responsible for the body’s reaction to allergens (Benson, 2015).

Aged black garlic effects were studied in rats for its benefits regarding cerebral damage and inflammatory response after ischemia. The study concluded that the black garlic had more than just antioxidant related beneficial effects on the brain. The aged garlic extract treatment diminished neurological alterations, as well as histological damages induced by cerebral ischemia. The treatment also proved to reduce the effect of post-injury related enzymes and proteins, and showed signs of aiding in inflammation (Ana Laura Colin-Gonzalez; Alma Ortiz-Plata; Juana Villeda-Hernandez; Diana Barrera; Eduardo Molina-Jijon; Jose Pedraza-Chaverri; Perla D. Moldonado, 2011).

Along the same line of researching neurological capabilities of black garlic components, another study focused on it effects against Alzheimer’s Disease. Alzheimer’s Disease, and other neurological degenerative diseases, are plaguing an aging society. Allicin, and other garlic components, have been found to help prevent some of the neurological effects of this disease (Paola Tedeschi; Manuela Nigro; Alessia Travagli; Martina Cantani; Stefania Merighi; Stephania Gessi, 2022).

The effects of black garlic and its potential for medical benefits is commonly researched. There are studies that showcase the effects of garlic and its various components in regards to fighting cancer. In one study, the authors focused on the various ways to ingest and consume
garlic and the potential benefits and components each particular preparation contributes to the consumer. There are various components that are prevalent in garlic that have been proven to aid in the processes involved in the battle of cancer. Garlic extracts have been shown to increase enzyme activity and inhibit the cancerous endothelial cells in mice. There is evidence that shows that garlic aid in reducing tumor growth in patients in regards to certain types of cancers such as skin and colon cancer. The antioxidants present in black garlic have been shown to prevent oxidative stress damage that can be caused by cancer cells. The various components found in garlic are discussed in regards to solubility and efficacy of consumption. There are beneficial components found in the various garlic products that are either water or fat soluble. This information helps relate specific information to bioavailability and utilizable components and benefits that are found in the different forms of garlic. (Zeinab Farhat; Pamela A. Hershberger; Jo L. Freudenheim; Manoj J. Mammen; Rachael Hagerman Blair; Diana S. Aga; Lina Mu, 2020).

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2.3 GOALS AND IMPLICATIONS

The goal of this research is to see which processing machine, duration of time, and temperature used will result in the highest antioxidant capacity. Time and temperature will be used to evaluate the antioxidant effects that the process has on the garlic. This will help create a foundation for further future research into black garlic.

The results from this experiment can help those at home as well as producers, doctors, patients, chefs, athletes and pharmaceutical companies to create a better product. The versatility and expansive benefits that this product encompasses can result in multifaceted uses and implications. The following explains how this research can potentially help just a few of the industries and businesses with the knowledge obtained from the end results. These are all either currently used methods, as well as theoretical implication and application of black garlic.

Pharmaceutical companies use aged black garlic extract in medications and supplements. This information could change the chosen production methods and provide them with more superior products that may yield more promising results. This could help expand their outreach and marketing platforms as well. There is even research that combining black garlic with methotrexate can create a more promising results due to the black garlic inhibiting the intestinal damage that methotrexate can cause in cancer patients (Tanvir Ahmed; ChinKun Wang, 2021).
The antioxidant properties of the black garlic can be made in the most effective way, making a superior product than products their competing companies are producing.

Doctors can recommend the preferred production method from this research to their patients. Those who experience inflammation and illnesses of various types may want to add black garlic into their diets as a supplement to aid in alleviating those symptoms. For example, those with Rheumatoid Arthritis may experience some pain relief from the symptoms that their disease causes. The same concept can be applied to almost any types of arthritic and inflammatory diseases. The information provided in this research can help those interested in adding it to their diets, and willing to take on the time-consuming project, without the trial-and-error period. They will be able to begin the project with the knowledge of the best home production method that will produce the highest number of antioxidants. This will allow them to introduce the most effective way to achieve the most beneficial and bioavailable form of black garlic into their system.

2.4 MATERIALS AND METHODS

SETUP

This experiment is a factorial design comparing time, temperature, and vessels as the variables being manipulated. The garlic was produced in various vessels that can either be found in the modern-day home, or restaurant. The vessels chosen could all be easily purchased through major companies, and online retailers. The garlic was produced using a sous vide, a fermentation station (a home proofing box), and a dehydrator.

All of the garlic obtained was purchase through Cuttler Produce, a local produce distributor. All garlic used in this experiment was hard-neck garlic, Morado varietal, harvested in Spain in July 2021. Once obtained, the garlic was vacuum sealed and stored in a -80°C freezer until ready.
to use. Heads of garlic were kept whole, and two (2) samples were made for each time and temperature adjustment, providing duplicate samples.

The temperature points that were assessed are: 57°C, 63°C, and 68°C. Each vessel was set to the same temperature and 8 raw heads of garlic were placed inside, with no additional preparation to the garlic themselves. The fermentation station and dehydrator had trays of water placed inside the vessel to help increase the humidity inside the machine to create a moisturized environment. These were refilled regularly throughout the process. While the garlic was fermenting, 2 samples were pulled at the following points in time: 25 days, 30 days, 35 days, and 40 days in order to provide duplicate assessment. The process was then repeated at each of the mentioned temperatures for all the cooking vessels. This rendered 36 samples, all of which were cooled, vacuum sealed, and then stored in a -80°C freezer until all samples were collected. The samples were collected as shown in Table 1. Each sample number had two (2) heads of garlic that correlated with the specific sample number, each of which was given a corresponding letter, either A or B, next to the sample number.

To evaluate statistical significance, Jasp software was used. The test performed were ANOVA, to compare significant difference in TEAC values between machine, time, and temperature. Tukey post-hoc test was performed to determine significance between all pairs of variables. Linear regression was performed to identify correlation between variables.
<table>
<thead>
<tr>
<th>BG Time &amp; Temp</th>
<th>57°C</th>
<th>63°C</th>
<th>68°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 days</td>
<td>SV: 1</td>
<td>SV: 13</td>
<td>SV: 25</td>
</tr>
<tr>
<td></td>
<td>DH: 2</td>
<td>DH: 14</td>
<td>DH: 26</td>
</tr>
<tr>
<td></td>
<td>FS: 3</td>
<td>FS: 15</td>
<td>FS: 27</td>
</tr>
<tr>
<td>30 days</td>
<td>SV: 4</td>
<td>SV: 16</td>
<td>SV: 28</td>
</tr>
<tr>
<td></td>
<td>DH: 5</td>
<td>DH: 17</td>
<td>DH: 29</td>
</tr>
<tr>
<td></td>
<td>FS: 6</td>
<td>FS: 18</td>
<td>FS: 30</td>
</tr>
<tr>
<td>35 days</td>
<td>SV: 7</td>
<td>SV: 19</td>
<td>SV: 31</td>
</tr>
<tr>
<td></td>
<td>DH: 8</td>
<td>DH: 20</td>
<td>DH: 32</td>
</tr>
<tr>
<td></td>
<td>FS: 9</td>
<td>FS: 21</td>
<td>FS: 33</td>
</tr>
<tr>
<td>40 days</td>
<td>SV: 10</td>
<td>SV: 22</td>
<td>SV: 34</td>
</tr>
<tr>
<td></td>
<td>DH: 11</td>
<td>DH: 23</td>
<td>DH: 35</td>
</tr>
<tr>
<td></td>
<td>FS: 12</td>
<td>FS: 24</td>
<td>FS: 36</td>
</tr>
</tbody>
</table>

**KEY:**

- **SV** = Sous Vide
- **DH** = Dehydrator
- **FS** = Fermentation Station

**PREPARATION**

After all the samples were completed and collected, they were defrosted and processed to prepare for the extraction protocol. All samples were kept at -80°C until this point, and they were defrosted to room temperature. The garlic was then peeled to remove the papery exterior skins, and then blended in a spice grinder until smooth. The various samples provided an array of textures that blended differently. For example, some samples were extremely dry and created a powder, while others were moister and created pastes. Each head of garlic was blended separately, with the grinder being washed and dried in between each sample. 1g of each sample was taken and placed in labeled 15 mL centrifuge tubes to be used in the extraction process, while the remainder of the samples were stored in labeled 50 mL centrifuge tubes and stored at -80°C.
EXTRACTION PROTOCOL
1g of the samples were mixed with 2.5 mL of a 4/1 acetone/water solution. The samples were then sonicated for 10 minutes, then centrifuged at 1000g for 10 minutes. The supernatant was collected into another container, and the remaining solids received another 2.5 mL of 4/1 acetone/water solution, sonicated for another 10 minutes, and again centrifuged at 1000g for 10 minutes. This second supernatant was then combined with the first and used for evaporation. This process was done with each of the samples prior to evaporation.

EVAPORATION (ISOLATION) PROTOCOL
The combined supernatants were placed in their own sections of a five (5) compartment spider flask in the rotary evaporator. The water tub was set to 40°C, and the spider flask was rotated at 20 rpm. The pressure for the evaporation process began at 307mBar, and remained there for approximately 30 minutes, or until there was no longer any acetone being pulled off the samples. The pressure was then decreased by 20mBar every 5 minutes until it reached between 160-170mbar, or was no longer able to reduce pressure, and no more acetone was being pulled off. The samples were then placed in labeled 15mL centrifuge tubes and stored at -80°C until all samples were finished evaporating.

A TROLOX EQUIVALENCY ANTIOXIDANT (TEAC) ASSAY
The next series of steps were performed in order to isolate and extract the phenolic components of the water-based antioxidants from the processed black garlic samples. The antioxidant potential was assessed and evaluated using a Trolox Equivalency Antioxidant Capacity (TEAC) analysis. This analysis compares the antioxidant potential of the processed black garlic samples to the known powerful antioxidant Trolox. The test includes the assessment of the slowing of the oxidative reaction rate on DPPH, which is used as the radical standard, and the sample’s ability to neutralize the radical compared to the standard curve of Trolox, thus
reporting the results as a “Trolox Equivalency” measure. A microplate reader was used to measure the absorbency loss at 517nm after 30 minutes of incubation at 27°C.

The first step to prepare the evaporated samples for the microplate reader was to take the volume of the supernatants for each sample, and 30% of each was taken and set aside to be used in the antioxidant process. Then a solution of water and formic acid at a 99.7/0.3 ratio was created by taking 60 µl of formic acid and adding it to 16 mL of water, and then diluting it up to 20 mL total solution with more water. The set aside 30% supernatant samples then received 0.3 mL of the water and formic acid solution. Then the samples were diluted with water until a volume of 3 mL total liquid was reached.

Next, a DPPH solution was made by mixing 8 mg of DPPH into a 200 mL solution of 80/20 methanol/water. This was sonicated for 4 minutes, then set aside until the Trolox solution was finished.

In order to dilute the Trolox solutions for varying comparison strengths, a 34 mL of a 1:1 acetone/water solution was made. 6 mg of Trolox was added to 16 mL of the 1:1 acetone/water solution to create a 1.5mM Trolox solution. Then the remaining acetone/water solution was used to dilute the Trolox at various rates to create a series of Trolox strengths that can calculate the effectiveness curve. The dilution amounts were as seen in Table 2:

Table 2:

<table>
<thead>
<tr>
<th>Sample #</th>
<th>1.5 mM Trolox Solution</th>
<th>PLUS</th>
<th>1:1 acetone/water Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0 mL</td>
<td>+</td>
<td>5 mL</td>
</tr>
<tr>
<td>T2</td>
<td>1 mL</td>
<td>+</td>
<td>4 mL</td>
</tr>
<tr>
<td>T3</td>
<td>2 mL</td>
<td>+</td>
<td>3 mL</td>
</tr>
<tr>
<td>T4</td>
<td>3 mL</td>
<td>+</td>
<td>2 mL</td>
</tr>
<tr>
<td>T5</td>
<td>4 mL</td>
<td>+</td>
<td>1 mL</td>
</tr>
<tr>
<td>T6</td>
<td>5 mL</td>
<td>+</td>
<td>0 mL</td>
</tr>
</tbody>
</table>
MICROPLATE ANALYSIS

A VersaMax Microplate Analysis was performed for this study. The absorbency was set to 517 nm and was set for a Kinetic measurement of 30 minutes at 27°C. The Trolox assessment samples were prepared in the microplate plate as follows: The sample blank was 10µL of 99.7/0.3 water/formic acid solution with 290µL of DPPH solution, 0mM Trolox was made by combining 10µL of T1 and 290µL of DPPH solution, 0.3mM Trolox Solution was made by combining 10µL of T2 and 290µL of DPPH solution, 0.6mM Trolox Solution was made by combining 10µL of T3 and 290µL of DPPH solution, 0.9mM Trolox Solution was made by combining 10µL of T4 and 290µL of DPPH solution, 1.2mM Trolox Solution was made by combining 10µL of T5 and 290µL of DPPH solution, 1.5mM Trolox Solution was made by combining 10µL of T6 and 290µL of DPPH solution. These were placed in the top row of the microplate to calculate the effectiveness curve of the Trolox.

The black garlic samples were placed across the microplate plate with 10µL of each diluted sample with 290µL of DPPH solution in each compartment of the plate. This was done for all 36 samples, both A and B were done separately, yielding 74 samples total for assessment.

2.5 RESULTS AND DISCUSSION
COMPARISON OF ANTIOXIDANT POTENTIAL ACCORDING TO PROCESSING PARAMETERS:

There are significant main effects of machine (p < .001), the TEAC values observed for the samples prepared by sous vide (21.82 uMol Trolox/g wet weight black garlic sample) were significantly higher than those prepared by dehydrator 12.35 82 uMol Trolox/g wet weight black garlic sample) and fermentation station (7.24 82 uMol Trolox/g wet weight black garlic sample), see Figure 1. Temperature was also significant on TEAC values (p = 0.004), samples prepared at 68°C (18.60 82 uMol Trolox/g wet weight black garlic sample) were significantly higher than
those prepared at 57°C (9.56 82 uMol Trolox/g wet weight black garlic sample), and those prepared at 63°C (13.23 82 uMol Trolox/g wet weight black garlic sample) were not significantly higher than those prepared at 57°C (9.56 82 uMol Trolox/g wet weight black garlic sample) (p= 0.325), and not statistically significant between those prepared at 63°C (13.23 82 uMol Trolox/g wet weight black garlic sample) and 68°C (18.60 82 uMol Trolox/g wet weight black garlic sample) (p= 0.096), see Figure 2. There was no significant effect of time (p = 0.832), and no significance in any combination of time comparisons (all p values >0.05), see Figure 3. There are also significant interaction effects between machine and time (p = 0.034).

There is a significant linear relationship between TEAC values and temperature (p = 0.006), but not with machine or time. The model with only temperature has an $R^2$ value of 0.102, meaning temperature explains 10.2% of the variance in TEAC values. The adjusted $R^2 = 0.089$, meaning temperature more accurately accounts for 8.9% variance in TEAC Values. There is a positive correlation between temperature and TEAC value, showing that for every 1°C increase in temperature, a prediction of an increase of 0.812 TEAC value can be expected.

In summary, the ANOVA showed significant main effects of machine and temperature. The post hoc tests indicated that there were significant differences between sous vide and fermentation station, as well as sous vide and dehydrator. for the machine factor, and between 57°C and 68°C for the temperature factor. There were no significant values within time levels. The linear regression analysis revealed a significant positive relationship between TEAC values and temperature.
2.6 CONCLUSION:
In regards to the machines, the data suggests that there is a significant difference in TEAC values between the dehydrator and sous vide, as well as between the fermentation station and sous vide. However, there is no significance between the dehydrator and fermentation station. In regards to temperature, the data suggests that there is only a statistically significant difference in TEAC values between 57 and 68°C. The data suggests that time has no significant effect on TEAC values. The linear regression data suggests that there is a positive predictive relationship between TEAC values and temperature. The data suggests that for every 1°C increased, there will be a TEAC value increase of 0.812.

2.7 STATISTICAL SUMMARY FIGURES (TEAC)
Figure 1: TEAC (uMol Trolox/g Wet Weight Black Garlic Sample) by Machine

<table>
<thead>
<tr>
<th></th>
<th>Dehydrator</th>
<th>Fermentation Station</th>
<th>Sous Vide</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAC</td>
<td>12.35 A</td>
<td>7.24 A</td>
<td>21.82 B</td>
</tr>
</tbody>
</table>

Figure 2: TEAC (uMol Trolox/g Wet Weight Black Garlic Sample) by Incubation Temperature (°C)

<table>
<thead>
<tr>
<th></th>
<th>57</th>
<th>63</th>
<th>68</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEAC</td>
<td>9.56 A</td>
<td>13.23 AB</td>
<td>18.60 B</td>
</tr>
</tbody>
</table>

Figure 3: TEAC (uMol Trolox/g Wet Weight Black Garlic Sample) by Incubation Time (°C)

<table>
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<td>TEAC</td>
<td>83</td>
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<td>13.51 A</td>
<td>12.44 A</td>
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</table>
CHAPTER 3: MANUSCRIPT II
PH AND BRIX ASSESSMENT OF BLACK GARLIC PRODUCTION
3.1 ABSTRACT

Black garlic is created by processing regular garlic at temperatures ranging between 60-90°C, with high humidity for upwards of one month. This end product yields a garlic that is a deep black color with a sweet flavor, pungent aroma, and soft malleable texture. Black garlic is increasing popularity in the culinary world with more products making it to market shelves.

PURPOSE: To assess the effects time, temperature, and cooking vessel has on PH and degrees Brix in the black garlic processing. METHODS AND MATERIAL: Garlic processed in each vessel at 57°C, 63°C, and 68°C for 25 days, 30 days, 35 days, and 40 days. Samples were diluted with distilled water and homogenized. A PH meter was used for PH assessment, and a refractometer was used for Brix assessment. RESULTS: The PH test resulted in a significant difference among temperature, time, and machine. For Temperature, the post-hoc tests show that of 57°C (5.01) was significantly higher than 68°C (4.74) (p < .001), 63°C (4.93) was significantly higher than 68°C (4.74) (p < .001), but not between 57°C (5.01) and 63°C (4.93) (p = .096). For time, 25 days (5.02) was significantly higher PH than 30 days (4.86) (p = .003), 25 days (5.02) was significantly higher than 35 days (4.87) (p = .006), 25 days (5.02) was significantly higher than 40 days (4.81) (p < .001). There was no significance found between 30 and 35 days (p = .995), or 30 and 40 days (p = .604), or between 35 and 40 days (p = .454). For machine, dehydrator and fermentation station, and sous vide (all p-values < .001). The fermentation station (5.15) showed significantly higher PH than both the dehydrator (4.87) and the sous vide (4.65). The dehydrator (4.87) showed significantly higher PH than then sous vide (4.65). It is predicted that for every 1°C increase in temperature, there will be a -0.024 decrease in PH, and for every 1 day increase in time, there will be a -0.013 decrease in PH. The Brix tests resulted in a significant difference among time, temperature, and machine. For temperature, the
post-hoc tests show that 57°C (52.81) was significantly lower than and 68°C (56.875) (p = 0.050), but not between 57°C (52.81) and 63°C (54.79) (p = 0.467), 63°C (54.79) and 68°C (56.875) (p = 0.431). For time, the post-hoc tests show that 25 days (52.94) was significantly lower than 30 days (58.19) (p = 0.013) only, but not between 25 days (52.94) and 35 days (53.75) (p = 0.784), 25 days (52.94) and 40 days (55.42) (p = 0.287), 30 days (58.19) and 35 days (53.75) (p = 0.114), or 30 days (58.19) and 40 days (55.42) (p = 0.480), or between 35 days and 40 days (p = 0.822). For machine, the dehydrator (83.96) was significantly higher than the fermentation station (51.04) (p < .001), the fermentation station (51.04) was significantly higher than the sous vide (29.48) (p < .001), and dehydrator (83.96) was significantly higher than the sous vide (29.48) (p < .001). The linear regression model for Brix had an R-squared value of 0.000, indicating that the model did not explain any of the variance in the data. CONCLUSION: The test results indicate that the effects time, temperature, and machine have on PH are all significant variables. The results also indicate a correlation between temperature and PH, as well as time and temperature on PH. The Brix results indicate that time, temperature and machine all had a significant effect on the degrees Brix. However, there was no definitive correlation found between the variables.
3.2 INTRODUCTION
PH AND BRIX INTRODUCTION

Black garlic is the result of processing normal garlic under heat and moisture for extensive periods of time. The process varies slightly depending on the producer, and will be discussed in more detail later on. The end results of creating black garlic yields a garlic that is a deep black color, sweet flavor, pungent aroma, and has a soft, malleable in texture. This product has had many uses and claims made towards it, some of which are unclear.

Black garlic, commonly referred to as fermented garlic, is an ancient ingredient originating somewhere in Asia, with several health claims behind it. Though the exact origin is unknown, for centuries it has been a prominent part of Korean, Japanese, and Thai cuisines (Katarzyna Najman; Anna Sadowska; Ewelina Hallmann, 2020). A controversial aspect of black garlic is whether or not it is truly a fermented product (Weissman, 2019). The normal processing temperature of black garlic is high for a ferment, but low for cooking. There are several reactions, such as the Maillard reaction, that occur, but only one recent study mentions the bacterial components present in black garlic (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018). That study is not widely known, and the chefs of the culinary world still argue about the truth of this.

Some chefs even claim that it is specifically not a ferment, and therefore should not be labeled as one (Weissman, 2019). The previously mentioned study proves otherwise. Another way of recognizing whether or not an item is fermented is by monitoring the PH levels throughout the process. If the PH lowers, then that indicates that an acid fermentation is occurring. Acid fermented vegetables are high in vitamins and minerals (Campbell-Platt, 2018).
Unlike salt fermented items, acid fermented vegetables reduce the growth of undesirable microorganisms by promoting good bacteria to produce high levels carbon dioxide and acid, which will rapidly lower the PH, killing off the bacteria that cannot tolerate the harsh environment. The bacteria that are naturally found in vegetables and could survive this type of environment are related to lactobacillus, and can even survive human digestive systems. These lactobacillus related bacteria found in acidic fermented vegetables also play a role in digesting and converting sugars in this process, which is the reason the degrees Brix was calculated in this experiment (Campbell-Platt, 2018).

The same chefs that claim fermentation is not going on within the process of making black garlic, seem of focus only on the presence of caramelization and the Maillard reaction (Weissman, 2019). Both reactions are notable and present in black garlic. Harold McGee describes caramelization as a complex series of reactions that involve the deepening and enrichment of sugars. This reaction uses sugar as a substrate, and tends to promote the formation of naturally occurring acidic compounds. The results are usually a sweeter tasting product with a darker color (McGee, 2004).

Though caramelization is responsible for some of the color change in black garlic processing, it is not the only color altering reaction. The Maillard reaction is another common reaction that occurs in making black garlic, though it provides more of black garlic’s signature black color than caramelization. This reaction’s substrates are both carbohydrates and amino acids that aid in developing flavor of color of the black garlic (McGee, 2004).

Unlike salt fermented items, acid fermented vegetables reduce the growth of undesirable microorganisms by promoting good bacteria to produce high levels carbon dioxide and acid, which will rapidly lower the PH, killing off the bacteria that cannot tolerate the harsh
environment. The bacteria that are naturally found in vegetables and could survive this type of environment are related to lactobacillus, and can even survive human digestive systems. These lactobacillus related bacteria found in acidic fermented vegetables also play a role in digesting and converting sugars in this process, which is the reason the degrees Brix was calculated in this experiment (Campbell-Platt, 2018). Another way of recognizing whether or not an item is fermented is by monitoring the PH levels throughout the process. If the PH lowers, then that indicates that an acid fermentation is occurring. Acid fermented vegetables are high in vitamins and minerals (Campbell-Platt, 2018).

Though there is controversy involving the status of black garlic being a fermented product, there is one article that was found to include and involve the microbial breakdown of black garlic, proving that it is a ferment. This research performed involved the characterization of the microbial community that developed during the black garlic processing. The importance of this research is not only within the classification of black garlic as a ferment, but the mapping of these microbes can indicate the metabolic potential available in black garlic (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018).

The study showed evidence of bacterial growth of various categories. The bacteria that survived the process were those that customarily survive acidic fermentation processes. The microbiome functional analysis of this study showed evidence of increased membrane transport, amino acid metabolism, and carbohydrate metabolism. This evidence indicates that these are the key microbial metabolic processes that are taking place during black garlic production (Zhichang Qui, Ningyang Li, Xiaoming Lu, Zhenjia Zheng, Mingjie Zhang, Xuguang Qiao, 2018).

The chemical components found in black garlic are dependent on the thermal processing procedure used to produce it. Certain sugars and amino acids required for the Maillard reaction,
but at high temperatures the rate of sugar reduction exceeds the reaction’s consumption rate. Though the higher temperatures reduce the processing time, it does compromise the sensory affects and flavor as described in this study. The roll sugar plays in formation of black garlic opens up the idea that lower temperatures for longer duration of time may be able to reduce the sugar loss and increase its presence in reaction consumption instead. Theoretically, the lower temperatures may yield a superior product (Shunsuke Kimura; Yen-Chen Tung; Min-Hsiung Pan; Nan-Wei Su; Ying-Jang Lai; Kuan-Chen Cheng, 2017).

3.3 GOALS AND IMPLICATIONS

The goals of this research are to see how processing machine, duration of time, and temperature used in processing black garlic will affect PH and degrees Brix. The PH and degrees Brix will also be calculated in this experiment in order to better understand the variables effects on the product. This will help create a foundation for further future research, and even production of black garlic. The PH will help determine food safety characteristics, while the degrees brix can potentially aid in predicting the desired sensory characteristics for producers in the food industry.

There are some culinary related black garlic items on the market. However, they are very costly and can easily impede on a restaurant’s food cost. There is also little information about the process used by these companies, which may in fact involve a step that could potentially render the health benefits useless. High end chefs also tend to prefer to make their elite culinary items in-house to ensure they are made properly and to their standards. This information will allow them to do that, along with complete control over their end product and utilization. The marketing aspects of making this product in-house are also extremely beneficial for the business itself. The PH and Brix assessments that were conducted can also aid chefs and producers in
altering the process in order to achieving their desired sensory characteristics in their end product.

As a former chef, this author has vast culinary knowledge of the implications of this research in the restaurant industry. This research will provide the knowledge of how to create the most beneficial black garlic product using equipment that most industrial kitchens or homes, either already have, or can be easily obtained. The knowledge from this research will provide the appropriate procedure to make this, which in turn will save chefs and home cooks time and money if they desire to utilize this product.

Pittsburgh Tribune launched an article about black garlic, and the popularity it was gaining back in 2009. Black garlic was patented by Scott Kim from South Korea in 2004, though it is known to have been around for hundreds of years prior to that. He planned on marketing the black garlic as a super food, and it took off in the culinary world due to its unique flavor (Kim, 2009). Chefs, such as Joshua Weissman, claim that the process of making black garlic is actually not considered a ferment, and only holds the presence of the Maillard reaction, which is why the garlic turns its signature black color. Though many processes happen in the formation of black garlic, the Maillard reaction is clearly not the only chemical reaction that could be going on. This confusion is something that needs clarification in order to provide the appropriate classification of what black garlic truly is to the culinary world. (Weissman, 2019).

3.4 MATERIALS AND METHODS
SETUP
This experiment is a factorial design comparing time, temperature, and vessels as the variables being manipulated. The temperatures being used range from 57-68°C. The garlic was produced in various vessels that can either be found in the modern-day home, or restaurant. The
vessels chosen could all be easily purchased through major companies, and online retailers. The garlic was produced using a sous vide, a fermentation station (a home proofing box), and a dehydrator.

All of the garlic obtained was purchase through Cuttler Produce, a local produce distributor. All garlic used in this experiment was hard-neck garlic, Morado varietal, harvested in Spain in July 2021. Once obtained, the garlic was vacuum sealed and stored in a -80°C freezer until ready to use. Heads of garlic were kept whole, and two (2) samples will be made for each time and temperature adjustment, providing duplicate samples.

The temperature points that were assessed are: 57°C/135°F, 63°C/145°F, and 68°C/155°F. Each vessel was set to the same temperature and 8 raw heads of garlic were placed inside, with no additional preparation to the garlic themselves. The fermentation station and dehydrator had trays of water placed inside the vessel to help increase the humidity inside the machine to create a moisturized fermentation environment. These were refilled regularly throughout the process. While the garlic was fermenting, 2 samples were pulled at the following points in time: 25 days, 30 days, 35 days, and 40 days in order to provide duplicate assessment. The process was then repeated at each of the mentioned temperatures for all the cooking vessels. This rendered 36 samples, all of which were cooled, vacuum sealed, and then stored in a -80°C freezer until all samples were collected. The samples were collected as shown in Table 1. Each sample number had two (2) heads of garlic that correlated with the specific sample number, each of which was given a corresponding letter, either A or B, next to the sample number.

To evaluate statistical significance, Jasp software was used. The test performed were ANOVA, to compare significant differences in values between PH, and degrees Brix by machine, time, and temperature. Tukey post-hoc test was performed to determine significance.
between all pairs of variables. Linear regression was performed to identify correlation between variables.

PREPARATION

After all the samples were completed and collected, they were defrosted and processed to prepare for the extraction process. All samples were kept at -80°C until this point, and they were defrosted to room temperature. The garlic was then peeled to remove the papery exterior skins, and then blended in a spice grinder until smooth. The various samples provided an array of textures that blended differently. For example, some samples were extremely dry and created a powder, while others had a moister consistency, and created pastes. Each head of garlic was blended separately, with the grinder being washed and dried in between each sample. 5g of each sample was taken and placed in labeled 50 mL centrifuge tubes, and 45 mL of distilled water was added to each. They were then sonicated for 10 minutes, and then blended with a handheld homogenizer.

PH ASSESSMENT

The pH of each sample was taken using a pH meter that was calibrated according to the manufacturer’s directions, prior to performing this experiment. The probe of the meter was cleaned between each sample recording with distilled water. The pH of each sample was then recorded.

BRIX ASSESSMENT

The Brix of each sample was taken by using a refractometer. The lens of the refractometer was cleaned between each sample recording using a clean paper towel and distilled water. The Brix was then recorded. The recorded degrees Brix was then calculated to the appropriate volume representation.
3.5 RESULTS AND DISCUSSION
VALUE COMPARISON OF PH ON PROCESSING PARAMETERS

The ANOVA table shows the results of a three-way analysis of variance for the variables of temperature (Degrees C), time (Days), and machine, and their interaction effects on PH.

The tests shows that all three main effects (temperature, time, and machine) are statistically significant (p < .001), indicating that each variable has a significant impact on the outcome variable.

The two-way interaction effects of temperature and machine, and time and machine are significant (p < .001 and p = .015, respectively), indicating that the effect of each variable on the PH depends on the level of the other variable.

However, the three-way interaction effect of temperature and time and machine is not significant (p = .625), indicating that the three variables do not interact in a significant way.

Post-hoc tests are conducted to investigate the differences between the levels of each variable. The results are adjusted for multiple comparisons.

For Temperature, the post-hoc tests show that of 57°C (5.01) was significantly higher than 68°C (4.74) (p < .001), 63°C (4.93) was significantly higher than 68°C (4.74) (p < .001), but not between 57°C (5.01) and 63°C (4.93) (p = .096), see Figure 5.

For time, the post-hoc tests show that there are significant differences between the time parameters. 25 days (5.02) was significantly higher than 30 days (4.86) (p = .003), 25 days (5.02) was significantly higher than 35 days (4.87) (p = .006), 25 days (5.02) was significantly higher than 40 days (4.81) (p < .001). There was no significance found between 30 days (4.86) and 35
days (4.87) (p = .995), or 30 days (4.86) and 40 days (4.81) (p = .604), or between 35 days (4.87) and 40 days (4.81) (p = .454), see Figure 6.

For machine, the post-hoc tests show that there are significant differences between the means of all combinations of machines. Dehydrator and fermentation station, and sous vide (all p-values < .001). The fermentation station (5.15) was significantly higher than both the dehydrator (4.87) and the sous vide (4.65). The dehydrator (4.87) was significantly higher than the sous vide (4.65), see Figure 4.

In conclusion, the post hoc test results indicated that the machine used and the temperature may have a significant effect on the mean scores, while the length of time the samples were processed did not. However, it is essential to note that some p-values were not adjusted for multiple comparisons, which may increase the risk of type I error. Therefore, further research is recommended to confirm these findings.

The linear regression shows the results of a regression analysis that explores the relationship between temperature (degrees C) and PH, and time (days) and PH.

Model 1 is the null model, which includes only an intercept. It shows that the intercept is statistically significant (p < .001) but the R-squared value is 0, indicating that the intercept alone cannot explain any variation in the PH.

Model 2 includes temperature as a predictor variable. It shows that temperature is statistically significant (p = 0.003) and the R² value is 0.117, indicating that temperature explains 11.7% of the variation in the PH. The adjusted R² value is 0.104 indicating that temperature more accurately explains 10.4% of the variance in PH, since the intercept has no meaningful value.
Model 3 includes temperature and time as predictor variables. It shows that both time and temperature are statistically significant (\( p = 0.044 \) and \( p = 0.003 \)) The R-squared value is 0.167, indicating that temperature and time together explain 16.7% of the variation in the PH. However, the adjusted R\(^2\) value is 0.143, indicating that time and temperature more accurately explain 14.3% variance of PH.

The results of the linear regression also indicate that temperature and PH, as well as time and PH, both have inverse predictive relationships with one another. It is predicted that for every 1°C increase in temperature, there will be a -0.024 decrease in PH, and for every 1 day increase in time, there will be a -0.013 decrease in PH.

**DEGREES BRIX COMPARISON ACCORDING TO PROCESSING PARAMETERS**

The ANOVA table shows the results of a three-way analysis of variance for the variables of temperature (degrees C), time (days), and machine, and their interaction effects on Brix. The table shows that all three main effects (temperature, time, and machine) are statistically significant at \( p < .001 \), indicating that each variable has a significant impact on Brix.

Post-hoc tests are conducted to investigate the differences between the levels of each variable. The results are adjusted for multiple comparisons. For temperature, the post-hoc tests show that 57°C (52.81) was significantly lower than and 68°C (56.875) (\( p = 0.050 \)), but not between 57°C (52.81) and 63°C (54.79) (\( p = 0.467 \)), 63°C (54.79) and 68°C (56.875) (\( p = 0.431 \)), see Figure 8.

For time, the post-hoc tests show that 25 days (52.94) was significantly lower than 30 days (58.19) (\( p = 0.013 \)) only, but not between 25 days (52.94) and 35 days (53.75) (\( p = 0.784 \)), 25 days (52.94) and 40 days (55.42) (\( p = 0.287 \)), 30 days (58.19) and 35 days (53.75) (\( p = 0.287 \)),
0.114), or 30 days (58.19) and 40 days (55.42) \( (p = 0.480) \), or between 35 days and 40 days \( (p = 0.822) \), see Figure 9.

For machine, the post-hoc tests show that there are significant differences between the means of all three machines. The dehydrator (83.96) was significantly higher than the fermentation station (51.04) \( (p < .001) \), the fermentation station (51.04) was significantly higher than the sous vide (29.48) \( (p < .001) \), and dehydrator (83.96) was significantly higher than the sous vide (29.48) \( (p < .001) \), see Figure 7.

The linear regression model for Brix had an R-squared value of 0.000, indicating that the model did not explain any of the variance in the data. The intercept term was significant \( (p < .001) \), but neither temperature (degrees C) nor time (days) were included as covariates in the model.

Overall, the ANOVA suggests that machine and its interactions with temperature (degrees C) and time (days) were the most important predictors of Brix. The post hoc tests suggest that there were significant differences between only one level of time (25 and 30 days), and one level of temperature (57-68°C), but all three machines showed statistical significance in regards to Brix. However, the linear regression model did not provide any meaningful information about the relationship between Brix and the covariates.

### 3.6 CONCLUSION

The data suggests that the machine was a significant variable in both the PH and Brix analyses. Time’s effect on PH was significant between 25 days and 30, 35, or 40 days. Though time was not significant between 30 days and 35, or 40 days, and also not significant between 35 and 40 days. Temperature showed significant effect on PH between 57 and 68°C, and between
63 and 68°C. However, 57 and 63°C were not significant when it came to PH. Linear regression showed an inverse predictive relationship between PH and time, as well as PH and temperature. Every 1°C increase is predicted to drop the PH -0.024. For every day the garlic is being processed, it can be predicted that the PH will drop -0.013. The only time pairing that showed significance in regards to Brix was between 25 and 30 days. The only temperature that showed significance was between 57 and 68°C. There was no predictive relationship between variables and degrees Brix.

3.7 STATISTICAL SUMMARY FIGURES FOR PH AND BRIX

Figure 4: PH by Machine

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<th>Fermentation Station</th>
<th>Sous Vide</th>
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<td>PH</td>
<td>4.87 A</td>
<td>5.15 B</td>
<td>4.65 C</td>
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Figure 5: PH by Incubation Temperature (°C)

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<th>68</th>
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<td>PH</td>
<td>5.01 A</td>
<td>4.93 A</td>
<td>4.74 B</td>
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Figure 6: PH by Incubation Time (°C)

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<td>PH</td>
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<td>4.86 B</td>
<td>4.87 B</td>
<td>4.81 B</td>
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Figure 7: BRIX by Machine

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<td>BRIX</td>
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<td>51.04 B</td>
<td>29.48 C</td>
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Figure 8: BRIX by Incubation Temperature (°C)

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<th>68</th>
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<tbody>
<tr>
<td>BRIX</td>
<td>52.81 A</td>
<td>54.79 AB</td>
<td>56.875 B</td>
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Figure 9: BRIX by Incubation Time (°C)

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<tbody>
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<td>BRIX</td>
<td>51.94 A</td>
<td>58.19 B</td>
<td>53.75 AB</td>
<td>55.42 AB</td>
</tr>
</tbody>
</table>
CHAPTER 4: CONCLUSION

In regards to the machines, the data suggests that there is a significant difference in TEAC values between the dehydrator being lower than the sous vide, as well as between the fermentation station being lower than the sous vide. However, there is no significance between the dehydrator and fermentation station. In regards to temperature, the data suggests that there is only a statistically significant difference in TEAC values between 57°C being significantly lower than 68°C. The data suggests that time has no significant effect on TEAC values. The linear regression data suggests that there is a positive predictive relationship between TEAC values and temperature. The data suggests that for every 1°C increased, there will be a TEAC value increase of 0.812. The data suggests that the machine was a significant variable in both the PH and Brix analyses. Time’s effect on PH was significant between 25 days having the highest PH and 30, 35, or 40 days, which had the lowest PH. Though time was not significant between 30 days and 35, or 40 days, and also not significant between 35 and 40 days. Temperature showed significant effect on PH between 57°C being higher than 68°C, and between 63°C being higher than 68°C. However, 57°C and 63°C were not significant when it came to PH. Linear regression showed an inverse predictive relationship between PH and time, as well as PH and temperature. Every 1°C increase is predicted to drop the PH -0.024. For every day the garlic is being processed, it can be predicted that the PH will drop -0.013. The only time pairing that showed significance in regards to Brix was between 25 days being significantly lower than 30 days. The only temperature that showed significance was between 57°C being significantly lower than 68°C. There was no predictive relationship between variables and degrees Brix.
CHAPTER 5: REFERENCES


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