EFFECTS OF STORAGE CONDITIONS ON α-ACID DEGRADATION ON INDIANA GROWN HOPS (*Humulus lupulus*)

by

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To my family and friends. Thank you for your support. Le Kamoso!

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TABLE OF CONTENTS

LIST OF TABLES 7
LIST OF FIGURES 8
ABSTRACT 11
CHAPTER 1. INTRODUCTION 12
1.1 Overall goals and specific objectives 13
CHAPTER 2. LITERATURE REVIEW 14
2.1 Scientific name and Botanical Features of Hops 14
2.2 Hop production in the World 16
2.3 Hop production in the United States of America 19
2.4 Hop Composition 19
2.4.1 Bittering compounds 20
2.4.2 Aroma Compounds 24
2.5 Hop Varieties 25
2.5.1 Chinook 25
2.5.2 Cascade 26
2.6 Analysis of hops 26
2.7 Hop Cone Processing 28
2.7.1 Hop Cones 28
2.7.2 Hop Pellets 28
2.7.3 Hop Extracts 29
2.7.4 Cryo Hops 31
2.7.5 Storage of Hops 31
2.8 Effects of Age on Hops 32
2.9 Degradation of α-and β-acids during storage 33
2.10 Hop Color 34
2.11 Current Industry Practices on Preventing Hop Oxidation 34
CHAPTER 3. MATERIALS AND METHODS 36
3.1 Hop Samples 36
3.2 Sample storage 36

3.3 Total α- acids and, total β-acids content and Hop Storage Index in hop pellets quantified by UV/Vis Spectrophotometry 38
3.4 Total α- acids and Total β-acids, humulones, and lupulones content in hop pellets quantified by HPLC-DAD 38
3.5 Hop Color Measurement 40
3.6 Total Oil Content by Steam Distillation 40
3.7 Moisture by air oven method 41
3.8 Kinetic modeling 41
3.9 Statistical analysis 42
CHAPTER 4. RESULTS AND DISCUSSION 43
4.1 Effects of Storage Temperature on hop α-acids Content 43
4.2 Effects of Temperature on β-acids 51
4.3 Effects of Temperature on Hop Storage Index 56
4.4 Effects of Storage Temperature on Oil Content 60
4.5 Effects of Temperature on Color 62
4.6 Degradation Rate Constants of α-Acids and β-Acids 71
CHAPTER 5. CONCLUSION AND RECOMMENDATIONS 82
REFERENCES 86

LIST OF TABLES

Table 2.1 World production of hops top 10 countries – 2017 18

Table 2.2 Composition of hops dry weight..... 20

Table 3.1 Sequence of sample testing per hop variety. Boxes marked with an X indicate when samples were retrieved for analysis 37

Table 4.1 Table showing moisture content and oil content of Hop pellets after 7 months of storage 62

Table 4.2 Average first order degradation kinetic constant (k) for total a-acids, cohumulone, n + adhumulone, total b-acids, colupulone, and n + adlupulone measured by HPLC for hop pellets stored at 4, 25, and 35^{o}C under air or nitrogen gas in the headspace. MSE is the mean square error of the data. Values represent average of duplicate analysis ± standard deviation. 80

Table 4.3 Activation energy and coefficient of determination for total α-acids, cohumulone, N + adhumulone, total β-acids, colupulone, N + adlupulone calculated from fitting experimental results with the Arrhenius equation. 81

LIST OF FIGURES

Figure 2.1 Cross-section diagram of a hop cone..... 15

Figure 2.2 Cone and parts: 1 – cone, 2 – covering bract, 3 – true bract, 4 – achene, 5 – cracked axis, 6 – cross section of a cone [a – cracked axis, b – covering bract, d – achenes] Source: (Rybacek 2012)..... 16

Figure 2.3 Hop growing regions in the world (Adapted from: http://www.worldatlas.com)..... 17

Figure 2.4 Molecular structures of α-acid homologs: Cohumulone, Humulone and Adhumulone (Adapted from: Schindler et al 2019) 22

Figure 2.5 Molecular structure of β-acid homolog: Colupulone, n-Lupulone and Adlupulone (Adapted from Schindler et al 2019) 22

Figure 2.6 Analogues of the α-acids and beta acids (Adapted from Briggs et al, 2004)..... 23

Figure 2.7 Conversion of Humulone to Isohumulone (Wikipedia) 24

Figure 4.1 Total α-acid content (%) of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric methods or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation. 47

Figure 4.2 Content (%) of a) Cohumulone and b) N + Adhumulone of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation..... 48

Figure 4.3 Total α-acid content (%) of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric method or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation. 49

Figure 4.4 Content (%) of a) Cohumulone and b) N + Adhumulone of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation..... 50

Figure 4.5 Total β-acid content (%) of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric methods or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation. 52

Figure 4.6 **Co**ntent (%) of a) Colupulone and b) N + Adlupulone of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation..... 53

Figure 4.7 Total β-acid content (%) of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric

method or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation. 54

Figure 4.8 Content (%) of a) Colupulone and b) N + Adlupulone of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation..... 55

Figure 4.9 HSI values for (a) cascade and (b) chinook hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation. 59

Figure 4.10 Total color difference (ΔE) for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation..... 65

Figure 4.11 L* values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation. 66

Figure 4.12 b* values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation. 67

Figure 4.13 a* values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation. 68

Figure 4.14 Hue angle (^{o}) values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation..... 69

Figure 4.15 Chroma values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation. 70

Figure 4.16 First order reaction correlation for degradation of total α-acids for Cascade hop pellets stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation..... 74

Figure 4.17 First order reaction correlation for degradation of total α-acids for Chinook hop pellets stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation..... 75

Figure 4.18 First order reaction correlation for degradation of cohumulone component for Cascade stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation..... 76

Figure 4.19 First order reaction correlation for degradation of N + adhumulone component for Cascade stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation. 77

Figure 4.20 First order reaction correlation for degradation of cohumulone component for Chinook stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation. 78

Figure 4.21 First order reaction correlation for degradation of N + adhumulone for Chinook stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation. 79

ABSTRACT

Hop (*Humulus lupulus* L) belongs to the Cannabaceae family and is well-known to be a dioecious, perennial climbing plant. The common hop is native to temperate climates, but due to its widespread commercial use in the brewing industry, hop plants are grown worldwide. The bittering components in hops, especially the α–acids, provide a pleasant bitterness and characteristic flavors to beer. On their own, α–acids do not contribute much to beer bitterness but do so after they are converted to iso-α–acids during the kettle boil step in brewing. β-acids in hops are not as important as α–acids, since they only supply about a third of the bittering power as a-acids but are mostly responsible for hops' antimicrobial properties. The goal of this study was to investigate the effects of storage conditions on the degradation rate of hop constituents. Pelletized hops from two varieties grown in Knightstown, IN were studied: Cascade (typically used as aroma hops) and Chinook (mostly used as bittering hops). The impact of storage conditions on hop chemical constituents, hop storage index (HSI), and color parameters was evaluated at three storage temperatures (4℃, 25℃, 35^{o}C) with hop pellets exposed to two different gases in headspace (nitrogen or air) for a duration of up to 168 days (4℃ and 25℃) and 70 days (35℃). Hop acids content was determined by UV/Vis spectrophotometry and HPLC-DAD. Results showed that increased storage temperature decreased the α–acid concentration in hops. At 4℃ and 25℃ the loss in α–acids was (14.5 – 23.4%) whereas at 35℃ there was a greater loss in α–acids. The α–acid loss between nitrogen flushed and air exposed pellets was not significantly different ($p>0.05$) except for Chinook pellets stored at 35℃. β-acids remained mostly stable for the duration of the study; however, at 35℃ the loss of β-acids over time became significant for both varieties. HIS values increased over time for both varieties at 4 and 25^{o}C, however, those values remained below the recommended 0.40 value. At 35^{o}C, the HSI values indicated unsuitable hops for brewing at the end of the study for both varieties. The color parameters L* and b* remained constant after 168 days at 4 and 25^{o}C, while the a* value and the hue angle showed a significant decrease with time and increasing temperature. At the conclusion of the study it was demonstrated that the loss in α–acids from Cascade pellets at 35℃ was lower than the loss in Chinook, suggesting that at high temperature the α-acids in cascade were more stable than those in Chinook.

CHAPTER 1. INTRODUCTION

Hops (*Humulus lupulus* L) are the main ingredient that contributes to beer's final aroma and flavor. The cone of the female hop plant contains lupulin glands, in which essential oils, resins, and bittering acids are found. The α-acids in the soft resin are responsible for making the beer bitter while the essential oils in the beer are responsible for flavor and aroma (Oladokun et al, 2016; Fandino et al, 2015). During brewing, hops are added at different stages: at various times throughout wort boiling and/ or at the end of it. At these two stages, addition of hops serves different functions: formation of bittering compounds when added early in boiling, or contribution of aromatic compounds when added at the end of boil (Rodolfi et al, 2019). Furthermore, hops can also be added to beer during fermentation in a process known as dry-hopping. A technique that is used to strengthen beer aroma by imparting oxidized α-acids (humulinones) into the beer which are reported in amounts of 0.2 – 0.5% w/w in hop leaves and pellets (Oladokun et al, 2016). For many years hops have been grown purposefully to supply brewers with one of the most distinctive ingredients in beer. This resulted in different cultivars and chemotypes with specific desirable properties for beer production (Matsui, Inui, Oka & Fukui, 2016; Preedy 2008). In addition to providing bitterness and unique flavors to beer, hops are also added in order to balance malt sweetness, increase foam stability, and enhance beer microbial stability.

Growing of hops in America started during the colonial times (Hop Growers of America, 2019). It is only of recent that hop growing in the state of Indiana started and it is still in a developmental stage. Generally, hops are usually dried and sold to brewers but, because of the small-scale operations in Indiana, most growers sell their hops fresh. Fresh hops can be used for seasonal brewing and usually have a very short shelf life. However, though there is a high cost for pelletizing hops, small scale growers are under increased pressure to dry and pelletize their hops to extend the shelf-life. In 2019 the largest grower of hops in Indiana completed the installation of a kiln drier in their farm, where hops are dried promptly after harvest, packed in bales, and stored under refrigeration temperature. Then, in December, when ambient temperatures have dropped sufficiently, hop bales are processed into pellets by the grower. Hops cones and pellets are usually stored in nitrogen-flushed or vacuum sealed mylar bags and stored at low temperatures to slow

down their deterioration (Ha, Atallah, Benjamin, Hoagland, Farlee and Woeste, 2017). Brewers usually prefer to use pelletized hops or hop extracts compared to whole hops because of reduced costs for transportation and storage, easier handling and consistent dosing when used in brewing. In order to preserve aroma compounds and avoid α-acid degradation hops should be stored at 0 – 5°C (Bamforth, 2006). When hops are exposed to higher temperatures, they age much faster compared to lower temperatures (Carpenter, 2014). Hop pellets that are nitrogen flushed before sealing and stored in refrigeration temperatures can last up to four years and five years if frozen (American Homebrewers Association, 2014). Though most brewers and growers know that hops should be stored at cold temperatures to last longer it is imperative to know the effects of temperature and storage conditions on hop pellets to determine the expected losses in valuable brewing compounds of hops during temperature abuse conditions. This study focuses on Indiana grown hops and how they are affected by storage conditions. Knowing this information will help hop growers in Indiana to store their hops in the right manner to preserve their brewing quality.

1.1 Overall goals and specific objectives

The overall goal of this project is to evaluate the impact of hop storage conditions at different temperatures and type of gas in package headspace on aging of pellets from two hop varieties grown in central Indiana in 2019. We hypothesize that the degradation rate of α-acids will occur rapidly under storage at intermediate (25^{o}C) and high (35^{o}C) temperature, and that the effect will be enhanced by the presence of oxygen in the headspace of the package.

In order to achieve the overall goal, the following specific objectives are proposed:

1. Evaluate the changes in hop α-acids, β-acids, hop storage index, color and oil content of hop pellets stored under various temperatures (4°C, 25°C, 35°C), package atmospheric composition (N_2, air) and time (0 to 6 months) of Cascade (aroma hop) and Chinook (bittering hop) pelletized hops
2. Determine the kinetic parameters order, rate velocity constant, and activation energy for degradation of hop α-acids in the Chinook and Cascade hop pellets.

CHAPTER 2. LITERATURE REVIEW

2.1 Scientific name and Botanical Features of Hops

Hop (*Humulus lupulus* L) belongs to the Cannabaceae family which includes the genus Cannabis, as well as genera previously classified in the Celtidaceae family. The common hop is well-known to be a dioecious and perennial climbing plant (Almaguer et al, 2014; Alonso-Estaban et al, 2019; Wang et al, 2008). There are three main northern temperate species representing the genus *Humulus*: *H. scandens* (Lourr.) Merr. (Japanese hop; syn. *H. japonicus* Siebold and Zucc.) and *H. yunnanensis* Hu originally found in temperate parts of Asia, as well as *H. lupulus* L. which is native to Europe, western Asia, and North America. Hops are classified into five taxonomic varieties: var. cordifolius in eastern Asia, var. lupulus in Europe and western Asia, var. lupuloides, neomexicanus, and pubescens in North America. The common hop plant is native to temperate climates, and due to its widespread use in the brewing industry, hops are commercially cultivated in various countries (Bocquet, Sahpaz, and Rivière, 2018).

Hops are perennial plants, with new growths emerging from the rootstock in the spring. The plants can produce a uniform crop for as long as twenty-five years if the plant is well maintained. Hops are also dioecious, which means that on a single plant they can produce a female or a male plant. Female plants are mostly preferred for cultivation as they are the ones that produce hop cones. Cones are known botanically as strobilus which have flowers that can be fertilized by the male pollen to produce a seed. Seeds, however, are not desirable in the cones as they have a high fatty acid content which can negatively impact foam and flavor stability in beer. The male plant is mostly needed when new hop varieties are being produced through crossbreeding (Biendl et al 2015). The cone of the female inflorescence of the hop plant is primarily used in beer flavoring. The cone consists of structures called bracts or bracteoles around the strig as illustrated in Fig 2.1. At their base the bracteoles are covered by lupulin glands that are formed as the hop ripens. These glands are only found in the female hop plant, a more detailed diagram of the hop cone and other parts is seen in Fig 2.2. The lupulin glands contain three main classes of secondary metabolites: essential oils, bitter acids and prenylflavanoids. These secondary metabolites are found in hops in different amounts and the amounts are used as an indication of how the hops are to be used,

whether as bittering or flavor and aroma hops (Wang et al, 2008). The bittering hops are usually those with the highest α-acid content while the aroma and flavor hops are those with the high essential oils content and lower α-acid content. The hop plant needs a minimum of 13 hours of light for growth to occur. If the amount of light in a day is less this may lead to plant dormancy. When the days are shortened the plant needs to produce 20 – 25 nodes before flowering, but when the days are too long flowering could be inhibited. In countries with less day hours such as South Africa and Kenya artificial light is used to increase the amount of light exposure. This delays flowering in plants and improve the crop yield (Briggs, Brookers, Stevens, and Boulton, 2004).

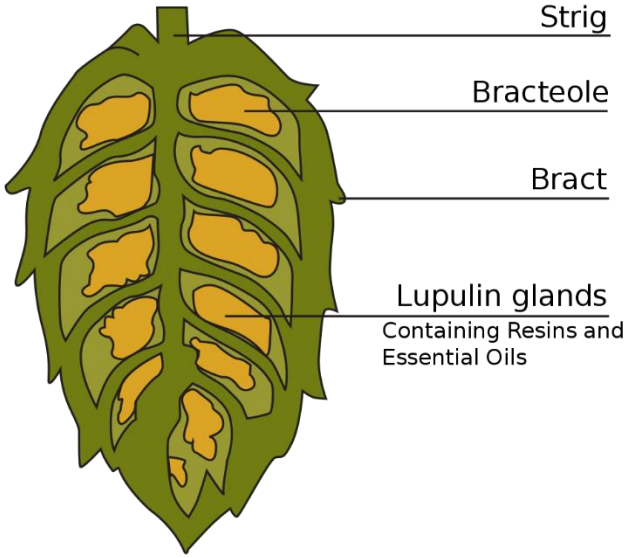


Figure 2.1 Cross-section diagram of a hop cone

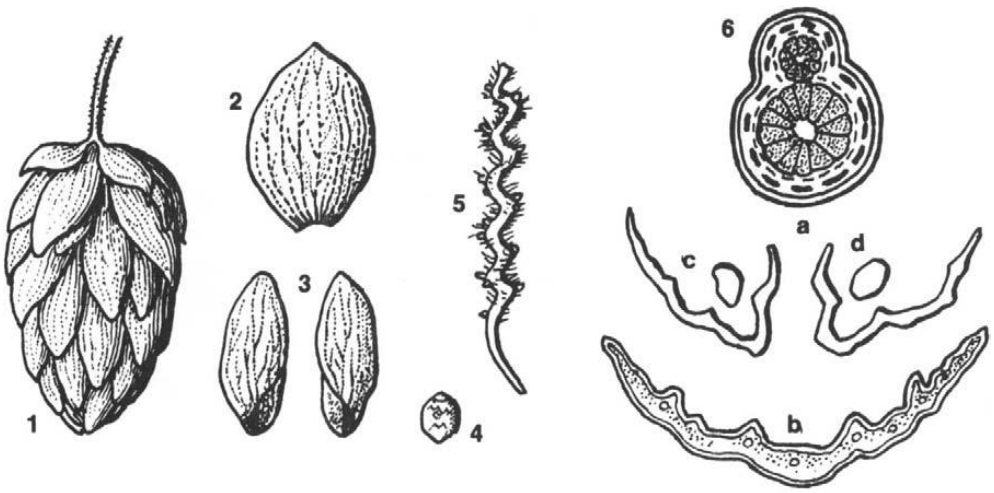


Figure 2.2 Cone and parts: 1 – cone, 2 – covering bract, 3 – true bract, 4 – achene, 5 – cracked axis, 6 – cross section of a cone [a – cracked axis, b – covering bract, d – achenes] Source: (Rybacek 2012)

2.2 Hop production in the World

The hop plant has been assumed to have originated in China, from where it then migrated to American and European countries (Alonso-Estaban et al 2019). In the world, hops grow best in the most moderate climate with fertile soils. Long days and warm summer temperatures are the specific climate requirements needed for hops to grow well. In addition, the amount of rain is also critical for effective cultivation of hops, which combined with specific climate requirements have created a reputation of hops being a very difficult plant to cultivate. In the Northern hemisphere hops grow well between latitudes 43° – 54° in Europe, 38° – 51° for North America, and 38° – 51° in Japan; whereas in the Southern hemisphere hops grow well in regions that fall within latitude 37° - 43° in Australia, 41° – 42° in New Zealand and 35° – 40° in Argentina (Verzele and Keukeleire 2013). The growing regions of hops in the world are shown in Fig 2.3. Examples of common hop varieties grown around the world include Cascade, Target, Saaz, Amarillo, Hallertauer Hersbruker, Newport, Nugget, and Spalt. Hop production in the world is led by two countries, Germany and USA as they are responsible for 75-80% of the global hop production. With respect to hop area under cultivation in the world, more than 60% belongs to those two countries. The specific regions with most of the hop production are the Hallertau region in Germany, and part of the Pacific Northwest region in the United States where hops are mostly grown in Washington, Oregon, and Idaho. Other countries in the world that grow hops include

Austria, Czech Republic, South Africa, Great Britain, and Slovenia (Almaguer et al, 2014). However, in 2017 hop production by metric tons was led by USA and Ethiopia, table 2.1 shows the top 10 countries of hops production in that year.

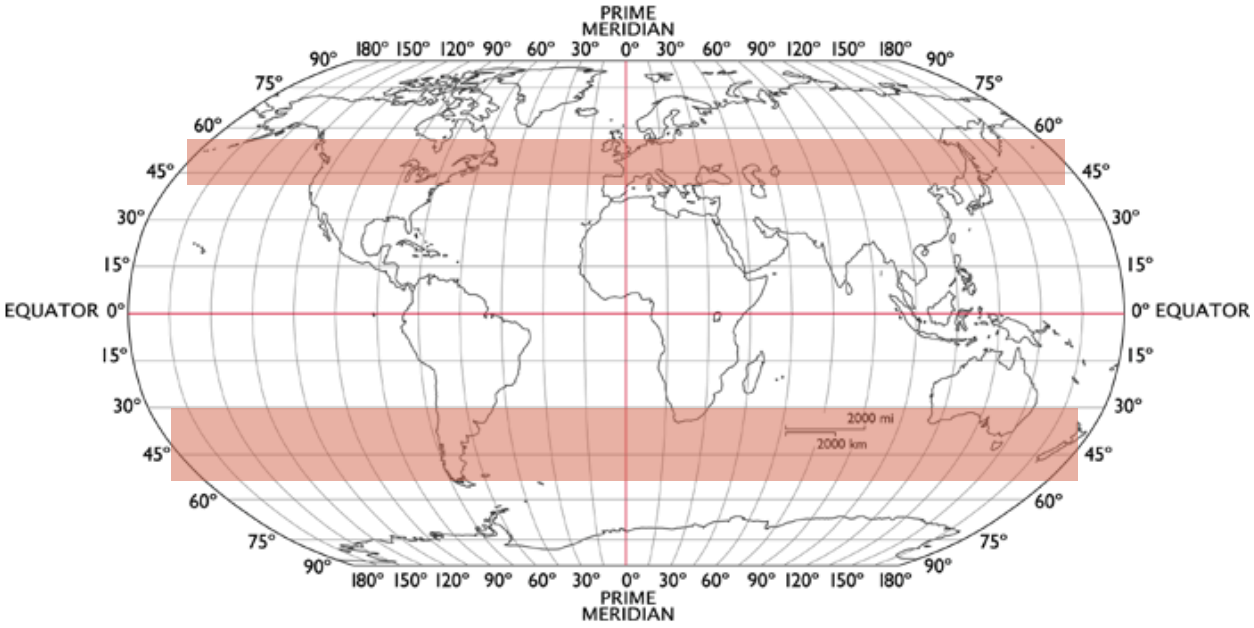


Figure 2.3 Hop growing regions in the world (Adapted from: http://www.worldatlas.com)

Table 2.1 World production of hops top 10 countries – 2017

Country	Production (metric tons)
United States	47,340
Ethiopia	38,418
Germany	32,582
China	6,822
Czech Republic	6,797
Poland	3,251
Slovenia	2,160
Albania	2,068
Korea, North	1,998
New Zealand	872

Source: FAOSTAT

2.3 Hop production in the United States of America

The first recorded commercial hop production in the United States of America dates back to 1648 in the Bay settlement of Massachusetts. Cultivation of hops then expanded to New York by the mid – 1800s making it the largest in hop production in terms of acreage (Hop growers of America, 2019). Part of the expansion in the US was due to England experiencing a series of crop failures leading to a stop in imports and brewers having to rely on locally grown hops (Brown, 2014). By the early 1900s, hop production spread to the pacific coastal states of California, Oregon and Washington, making these states the major producers of the hop crop in the United States. Suitable climatic conditions in the Pacific Northwest with fertile soil, abundant irrigation water and dedicated multigenerational farms along with excellent post-harvest storage and processing facilities has resulted in the production of high-quality hops desirable for the brewing sector (Hop growers of America, 2019). Though the major states that grow hops in the United States are Washington, Oregon and Idaho, hops are being grown in every state of the country. The hops are grown as far as south Florida, and even though their growing in acreage is measured in tens of acres and not in thousands, they still cater for local craft brewers. Most hop farming occurs in a narrow latitude of 44° – 51°, covering a lot of the country including states like Michigan and New York with hop growing histories (Grossman, 2020). Some of the top hop varieties grown in the United States from 2010 to 2015 are Cascade, Centennial, Chinook, Simcore, Citra, Amarillo, Crystal, CTZ, Willamette, Ahtanum, and US Golding (Brophy, 2016).

2.4 Hop Composition

Hop cones have many chemical components, but not all of them are important in beer making. However, the composition of hops is important in brewing and can influence the quality of beer (Kunze, 2004). The composition of the hop by weight is shown in Table 2.2. The lupulin resins can be divided into two major groups: the soft resin and the hard resin. The soft resin fraction contains most of the substances that contribute to the bitterness of beer, of which the α-acids are the most important components (Rybáček, 2012). The brewing value of the hop is mostly determined by the content of the soft resin and essential oils that are only slightly soluble in water. A-acids have been characterized into five different homologues being α-bitter acids (humulones), β-bitter acids (lupulones), γ-bitter acids (humulinones), δ-bitter acids (hulupones), and ε-bitter

acids which have not been specified yet. A-acids are the most bitter acids of the bittering acids, when oxidized β-acids form hulupones which are bitter and contribute 30 – 50% to the final bitterness of beer (Rybáček, 2012). It was shown that resins contain 20 – 25% water soluble constituents that dissolve in the boiling wort, these constituents are carbohydrates, amino acids, protein, polyphenols, and inorganic salts (Briggs et al, 2004). Furthermore, 2% of sugars mainly consisting of glucose, fructose and raffinose are contained in hops including 1 – 2% of pectin (MacWilliam, 1953). Briggs et al., (2004) further went on to mention that nitrogen in hops is contained in proportions of 2.0 – 3.5% which is equal to 12.5 – 21.7% protein, of which 0.5% nitrogen (3.1% protein) is soluble in water. Also contained in hops is 0.1% amino acids and 8% ash (inorganic matter) in dry hops, the rest of the composition includes polysaccharides and lipids which include oil, fats and waxes which are not soluble in water.

Table 2.2 Composition of hops dry weight

Components	Composition (%)
Bitter substances	18.5
Hop oil	0.5
Polyphenols	3.5
Protein	20.0
Minerals	8.0
Lipids	32.0

Source: Kunze, (2004), Briggs et al., (2004)

2.4.1 Bittering compounds

There are two main types of bitter acids in hops, the α-acids and the β-acids. The α-acids (humulones) which are found in the soft resin fraction are the most abundant in hops and are responsible mainly for bitterness in beer, especially after they have been isomerized to iso-α-acids (isohumulones) during wort boiling (Lafontaine et al, 2019). α-acids are responsible for the characteristic bitter taste and have three characteristic homologs which are: humulone,

cohumulone and adhumulone (Fig 2.4). β-acids also known as lupulones are not as bitter as α-acids and are insoluble in wort and lost during boiling. Just like α-acids they also have three homologs: lupulone, colupulone and adlupulone (Fig 2.5). β-acids are present in lower concentration than α-acids and the β-acid to α-acid ratio in hops is variety specific (Roberts, 2016). Fig 2.6 shows the molecular structure of all the homologs of α-acids and β-acids. A-acids content is highly dependent on the hop variety, usually on average the α-acid content of hops is between 9 – 10wt%, but recently developed varieties may contain up to 19 wt% (Fandino et al, 2015). The conversion of α-acids to iso-α-acids during wort boiling occurs in a process known as thermal isomerization as shown in Fig 2.7. This process is important in brewing because iso-α-acids impart bitterness in beer as α-acids are not bitter. Compared to quinine, which is a reference compound for bitterness comparison, iso-α-acids are almost equally bitter, thus iso-α-acids are strongly bitter. In addition to providing bitterness iso-α-acids help to stabilize beer foam and provide anti-microbial activity as they inhibit the growth of gram-positive bacteria (De Keukeleire, 2000). The hop value is determined by the content of α-acids and hence the importance in their preservation. With respect to composition of α-acids, low cohumulone varieties are preferred. Ideally, the proportion of cohumulone should be less than 20% of the total α-acids. The reason for this is because the bitterness provided by cohumulone has been described as “harsh” bitterness which is undesirable in contrast to the desirable bitterness derived from humulone and ad-humulone described as “fine” bitterness. However, the high value attributed to α-acids has resulted in the development of high α-acid varieties that have been grown more often in the recent decades, specifically those with α-acid content of 12 – 16% but with low cohumulone content (Kunze, 2004).

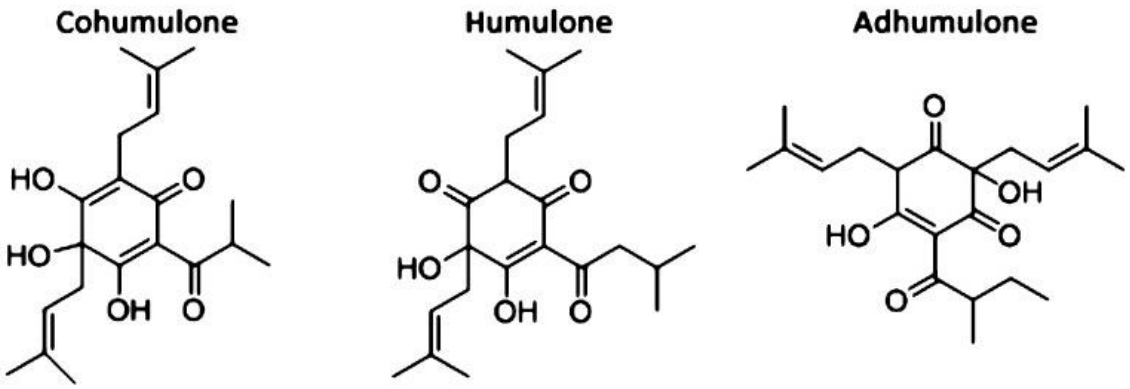


Figure 2.4 Molecular structures of A-acid homologs: Cohumulone, Humulone and Adhumulone (Adapted from: Schindler et al 2019)

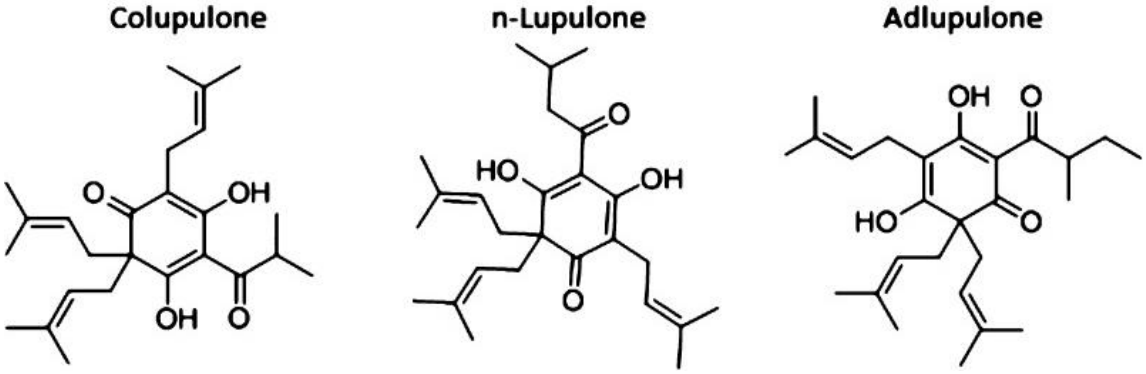


Figure 2.5 Molecular structure of β-acid homolog: Colupulone, n-Lupulone and Adlupulone (Adapted from Schindler et al 2019)

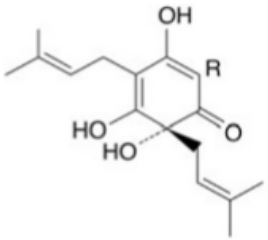
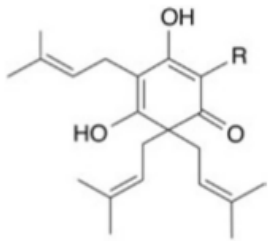
		α-acids					β-acids		
									
	Acyl side chain (R)	Name	Formula	m.p. (°C)	$[\alpha]_0^{24}$	pKa	Name	Formula	m.p. (°C)
a	-CO.CH_2.$CH(CH_3)_2$ isovaleryl	Humulone	$C_{21}H_{30}O_5$	64.5°	−211°	5.5	Lupulone	$C_{26}H_{38}O_4$	92°
b	-CO.$CH(CH_3)_2$ isobutyryl	Cohumulone	$C_{20}H_{28}O_5$	oil	−208.5°	4.7	Colupulone	$C_{25}H_{36}O_4$	93−94°
c	-CO.$CH(CH_3)$.CH_2.CH_3 2-methylbutyryl	Adhumulone	$C_{21}H_{28}O_5$	oil	−187°	5.7	Adlupulone	$C_{26}H_{38}O_4$	82−83°
d	-CO.CH_2.CH_3 propionyl	Posthumulone[a]	$C_{19}H_{26}O_5$	oil	–	–	–[d]	$C_{24}H_{34}O_4$	101°
e	-CO.CH_2.CH_2.$CH(CH_3)_2$ 4-methylpentanoyl	Prehumulone[b]	$C_{22}H_{32}O_5$	oil	−172°	–	–[e]	$C_{27}H_{40}O_4$	91°
f	-CO.$(CH_2)_4$.CH_3 hexanoyl	Adprehumulone[c]	$C_{22}H_{32}O_5$	–	–	–	–[e]	$C_{27}H_{40}O_4$	90°
g	-CO.CH_2.CH_2.$CH(CH_3)$.CH_2.CH_3 4-methylhexanoyl	–	$C_{23}H_{34}O_5$	–	–	–	–	$C_{28}H_{42}O_4$	91°

Figure 2.6 Analogues of the α-acids and beta acids (Adapted from Briggs et al, 2004)

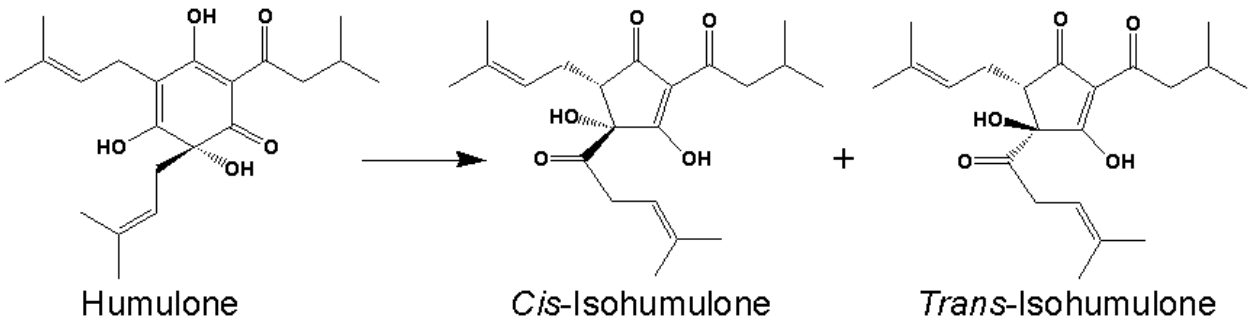


Figure 2.7 Conversion of Humulone to Isohumulone (Wikipedia)

2.4.2 Aroma Compounds

Hops contain essential oils that impart special aromas to beer. The number of chemical compounds in hop essential oils have been identified to be close to 1000 different ones that can be easily isolated by steam distillation (Steenackers, De Cooman & De Vos, 2015; Eyres, Marriott & Dufour, 2007). Unlike the hop resins which provide bitterness, essential oils, which make up between 0.5 and 3% (v/w) of the whole hop cones, are the main contributors to the aroma and flavor of beer. Much of the mass of the oils is made up of terpene and sesquiterpene hydrocarbons. The cyclic sesquiterpenes which include α-humulene and β-caryophyllene, depending on the hop variety, have mass ratios of 5 – 45% and 4 – 20% respectively making them the most dominant of the oils. According to Rettberg (2018), myrcene is the important volatile in hop varieties. Its concentration in hop dry matter ranges from 3 – 10 mg/g. Myrcene contributes resin, piney and herb-like odors, while α-humulene and β-caryophyllene both have spicy and woody odor. It was initially believed that hop essential oil profile depended on variety, but more specifically the amounts of hydrocarbons and oxygenated compounds vary according to variety and also hop age. It was later discovered that there were compositional differences in essential oils within varieties. Some components in similar varieties are affected by the growing conditions and growing regions. In certain varieties specific components may be found in minimal amounts or be completely absent. Furthermore, hop oil composition varies from year to year within the same variety (Almaguer et al, 2014).

2.5 Hop Varieties

In the world there is a great amount of distinctive hop varieties that are cultivated in specific growing regions and countries (Van Holle et al, 2017). The selection of hop varieties when growing and selling is important as hops are a vital and the most expensive ingredient in beer production (Kunze, 2004). The Germans classify hop varieties as aroma or bittering hops because they believe that their traditional hops are more aromatic than newer varieties. With that aroma hops were kept for late addition during wort boiling or dry hopping. Bittering hops are usually added at the beginning of boiling so that any undesirable volatiles would be evaporated (Briggs et al., 2004). In addition to this classification Kunze (2014), divided hop varieties into three groups: aroma, bittering and high α-acids varieties. Aroma varieties are known for their pleasing hop aroma, low cohumulone content and a low α-acids content of 2.5% - 5%. These varieties include Saaz, Hallertau, Hersbruck, Cascade, Mt Hood, Perle, Spalter Select and Hallertauer Traditional (De Cooman, Everaet and De Keukeleire 1998; Kunze 2004). High α-acid varieties (Northern brewer, Wye Target, Nugget) contain high proportions of cohumulone, but typically not more that 25%. The bittering varieties are recognized by having α-acids content between 10 and 15%, these varieties include but are not limited to Kent, Cluster and Brewers Gold. Low cohumulone varieties, usually less than 20% of total α-acid, are preferred as they have a reputation of providing a softer and more pleasing bitterness (Kunze, 2004). Recently hops are being grown in Indiana and with 21 hop farms in the state, some of the varieties grown are Chinook, Cascade, Centennial, Crystal, Comet, Michigan Copper, Nugget, Zeus, Wild, Brewers Gold, Fuggle, New port, Glacier, CTZ, Triple Perle, Tahoma, Cashmere, Columbia, Mackinac, Mt Hood, Pearl, Bitter gold, Galena, and Columbus (Indiana Hop Growers Association, 2019). In this study, Chinook and Cascade varieties were selected for evaluation.

2.5.1 Chinook

In 1985 Chinook was bred to be a bittering hop but has been lately recognized as a dual-purpose hop and is suited for brewing many beer styles. Its spicy and piney characteristics transfer into beers' flavor and aroma, and it also contributes grapefruit notes that emphasize its bitterness (Yakima Valley Hops 2018). The chinook hop comes from crossing two types of hops, Peltham Golding and USDA male 63012M (Bamforth, 2006; Hieronymus, 2012). Chinook hops had an

average of 12% and 3.5% α- and β-acids respectively after being tested as selection W421 – 38 for a period of 9 years in Washington in 1974. In 1980 selection W421 – 38 was tested in Washington and Oregon and averaged α-acids at 13% and β-acids at 3.8%. When tested again between the years 1982 to 1984 α-acids concentration averaged 14.1%. Its α-acid content shows a majority of humulone (60%), followed by cohumulone (31%), and adhumulone (9%). In dried form Chinook hops have an average of 1.6% oil content and the major components of the oil include 40% myrcene, 20% humulene and 9% caryophyllene (Kenny and Zimmerman 1986). Chinook hops also contain the compounds butyrolactone, 2-furanmethanol, linalool, geraniol, n-decanoic acid that are highly important for dry-hopping beer and an unknown compound that causes a musty aroma in beer. The compounds found in the least amounts but that still contribute to the flavor and aroma were heptanoic acid and two other unknown compounds that contribute a fruity aroma (Vollmer, Lafontaine and Shellhammer 2018).

2.5.2 Cascade

According to Rodolfi et al. (2019), Cascade hops belong to the aroma hops varieties and are characterized by a citrusy and fruity aroma. They have a total α-acids content in the range 4 – 7%, of which 30-40% is cohumulone, a β-acids content of 4.8-7% and total oil content of 0.7-1.4%, in which myrcene represents 40-60% of the total amount. Cascade hops are the lead aroma hops cultivated in the USA. The dominant component in the essential oil is myrcene (50 – 60%) which accounts for its citrusy aroma. Other aromas described in the Cascade variety include spicy, floral, woody and earthy notes. These are owed to other non-dominant essential oils present in this cultivar such as α-humulene, caryophyllene and β-farnesene. The floral and fruity aromas are mostly due to the presence of linalool (Nance and Setzer 2011). It was found that among other oil compounds in Cascade powdered hop pellets, linalool and geraniol were important for flavor because of their high flavor dilution (FD) numbers of 1024 and 128 respectively (Vollmer, Lafontaine and Shellhammer 2018).

2.6 Analysis of hops

Over the years many methods have been used to quantify components in hops. In the US, the American Society of Brewing Chemists (ASBC) is the organization that approves and publishes

the standard methods of analysis for beers and beer ingredients. For hop analyses, the use of High Performance Liquid Chromatography (HPLC) has been adopted commercially for the quantification of α-acids, β-acids and iso-α-acids, but ASBC spectrophotometric method (ASBC Hops – 6) and EBC lead conductance titration are still widely in use. The use of lead conductance method was a replacement for a more gravimetric method that was complex, this gravimetric method was described by Ford and Tait in their study in 1924 (Moir, 2000). The reaction for lead acetate in the EBC is not specific for α-acids hence the outcome is given as Lead Conductance Value (LCV). When the hops are fresh the LVC gives an accurate measure of the α-acids content in hops compared to hops aged during storage as the products of oxidation react with the lead acetate. The light absorption of α-acids and β-acids under both alkaline and acid conditions was studied by Alderton et al (1954). Specifically, the absorbance of the alkaline solution was measured at 275nm (minimum wavelength for both α- and β-acids), 325nm (maximum wavelength for α-acids) and at 355nm (maximum wavelength for β-acids), which led to the development of regression equations for estimation of α-acids and β-acids content in hop samples. This method was later adopted by the ASBC and was the first method to quantify %β-acids (Briggs et al, 2004). Likens et al, (1970) used the method by Alderton et al (1954) to propose that the ratio of absorbance readings at 275 and 325 nm (A_{275}/ A_{325}) produced a value called hop storage index (HSI) and that a value of 0.24 indicated fresh hops whereas a 2.5 value indicated entirely oxidized lupulin, this method was also adapted by the ASBC for calculation of the HSI. With the use of HPLC, it has been internationally accepted that hop extracts be used as calibration standards for the measure of α and β-acids (American Society for Brewing Chemists, 1999). For quantification of essential oils in hops steam distillation has been used, by passing steam through ground hops for 4 hours and then removing the oil from the condensate by extraction with ether (Green and Osborne, 1993). However, in the ASBC method essential oil is collected in a graduated trap during steam distillation for 4 – 7 hours and the collected oil volume measured after distillation. Gas chromatography mass spectrometry (GC – MS) was found to be very valuable in determining the main components of hop essential oils such as monoterpenes and sesquiterpenes. These comprise humulene, bisabolene, caryophyllene, farnesene and elemene skeletons. Retention parameters and m/ z values for molecular ions for selected compounds from hop essential oils are needed to use this method. The use of distillation before GC-MS analysis serves as an advantage as the oils

obtained will be ready to be used without the need of additional purification (Knez Hrnčič, Španinger, Košir, Knez, and Bren, 2019).

2.7 Hop Cone Processing

After harvesting, hop cones need to be rapidly processed to avoid spoilage and oxidation reactions that will turn the hops brown and produce undesirable odors. Hop pellets and hop extracts are most of the hops processed today (Bamforth, 2006). Hops are processed into natural hops (15-20%), hop pellets (40-45%), hop extracts (30%) and isomerized products (10%) on a global basis (Kunze, 2004). Depending on the number and the type of operations used, various products can be obtained as described next.

2.7.1 Hop Cones

Originally the hops that were used for brewing were added as dried whole hop cones. The cones are removed from the bines, cleaned and conveyed into a walk-in drying kiln that uses blowers and heaters to ensure controlled drying of the cones to a moisture content between 8 and 10% (Brown, 2013). Once the hops were dry, they were compressed into hop bales and transported to breweries. However, the storage conditions of hop cones were not ideal, and were easily affected by storage time and temperature. Uncontrolled storage conditions compromise the quality and flavor of hop cones and lowers the utilization rate to as low as 30% or less. For this reason, many brewers decided to use hop pellets and extracts as they are cheaper to transport and store, easier to handle and yield more consistent utilization efficiency (Reeb-Whitaker and Bonauto 2014; Schonberger, 2006). Nevertheless, some craft breweries may still use dried whole hop cones from time to time.

2.7.2 Hop Pellets

More than a century ago it was discovered that the amount of bitterness in beer could be increased during wort boiling if the hops were added in a ground form. Though studies showed that there was an increase in acid utilization of up to 20% when using ground hops, brewers were very reluctant to use them until the invention of the whirlpool separator as it made it easy to get rid of spent hop powder during brewing (Moir, 2000). There are two types of hop pellets that are

commonly used in brewing: type 90 and the type 45 hops. Type 90 pellets indicate that approximately 90% of the original bale mass is in the pellet; whereas type 45 hops are defined as pellets that are enriched before being made into pellets, and where about 55% of the hop material is lost. Of these two types, type 90 pellets are more common. Before being dried to 6 – 8% moisture hop cones are cleaned. They are then milled to form a powder and fed to a pellet mill where the powder will be compressed and forced through a die. The temperature during pellet production is raised by the shear forces and friction generated during the process. Therefore, to ensure that heat does not degrade the α-acids, carbon dioxide in the form of dry ice is added prior to pelleting to keep the powder cool. Pellets are packaged in laminated bags that are flushed with an inert gas prior to being heat sealed. When pellets are vacuum packed are usually flushed with an inert gas, typically nitrogen, to prevent pellets from adhering to each other. Exposure to atmospheric oxygen is also minimized as this may compromise the α-acids and essential oils in the hops both of which are essential in the brewing process (Hughes and Simpson, 1993).

2.7.3 Hop Extracts

Hop extracts are the products obtained after the concentration, by physical means of the desirable components of hop cones such as α-acids and essential oils. Solvent extraction has been done for a very long time and has aided in the understanding of the major resin of the plant cone and its assistance in improving beer flavor (Clarke 1986). Various solvents such as ethanol, dichloromethane, benzene, methanol, hexane and trichloroethylene, have been used in the extraction process but have raised concern from consumers. More recently, liquid carbon dioxide and supercritical carbon dioxide have been used to obtain hop extracts, which is much safer and more effective than the traditional solvents used in the past. Both types of carbon dioxide extractions are known to produce a high concentration of α-acids. Liquid carbon dioxide extraction produces α-acid contents of 30 – 60% whereas supercritical carbon dioxide can result in α-acid content of 27 – 55% (Hughes and Simpson, 1993). The former produces a product with less unwanted residues such as chlorophyll and hard resin than the latter. The use of liquid carbon dioxide is more selective but presents a disadvantage with regards to the time taken during the process as this process takes several hours. Though using supercritical carbon dioxide would be much faster it is unfortunately very expensive. The use of hop extracts is more advantageous as

the extracts are concentrated to a specific amount of α-acids, meaning that there is more control on hopping rate. The use of hop extracts eliminates the need to use hops and to deal with separation of the liquid and spent hops, the use of extracts also is more convenient as they have a better shelf life and for this reason are more economic as there will be less degradation and less need to buy more (Hughes and Simpson, 1993; Srečec, Rezić, Šantek, and Marić, 2008).

2.7.3.1 Isomerized Hop Extracts

It is possible to isomerize hop extracts, and these isomerized products can be used at different stages during brewing. In this form, extracts with higher utilization rate of 95%, compared to 25 – 30% utilization rate of cones and pellets, can be used as most of the intermediate products will be precipitated during brewing (Kunze 2004). Moir (2000) reported that in 1959 it was discovered that isomerization yields could be significantly increased under alkaline conditions and that the iso-α-acids that were obtained could be added to beer post-fermentation, with utilization improvement. A purification step is necessary before or after isomerization to prevent contamination by unwanted residues, heavy metals and toxic solvents that could end up in the beer. Isomerized hops keep well at temperatures as low as 5°C and when kept in unopened containers remain stable for a very long time. Isomerized hops are also easier to transport, and store compared to hop pellets and cones as they are more stable (Hughes and Simpson 1993; Kunze 2004 & Moir 2000).

2.7.3.2 Specialty Hop Extracts

Specialty hops products are defined as those that have been modified with organic materials to cause a more rapid or complete isomerization of α-acids or hop oil addition or incorporation that is beyond the normal amounts that should be present (Clark 1986). Powdered extract is formed when hop extracts are combined with silicic acid. Powdered extracts have the advantages of being more stable than liquid extracts, can be handled with ease, and improve the colloidal stability in finished beer (Hughes and Simpson 1993). Clark (1986) furthermore stated that it would be more convenient to produce extracts where the α-acids were completely isomerized than partially isomerized since α-acids could combine with other organic materials or hop components that would compromise flavor quality of the beer, especially when added post fermentation. Hughes and Simpson (1993) explained that the removal of α-acids from extracts would leave behind what

is known as base extracts which contain β-acids, other resin materials and some hop oils. These extracts, without α-acids can be used to prevent excessive foaming in the brewing kettle and can be used to impart a specific aroma in the wort kettle. Base extracts are good for use in light stable beers because they do not contain α-acids or their residues. Base extracts have antimicrobial properties especially when bittering is done post-fermentation (Bamforth, 2006).

2.7.4 Cryo Hops

Cryogenic hop lupulin has a high concentration of α-acids and essential oils that are mainly extracted from the lupulin constituent of the hop cone. The production of the enriched pellet is associated with cryogenic methods and mechanical methods. The high lupulin content pellet is produced under a high nitrogen-low oxygen environment at temperature between 0°F and - 50°F (-17.8°C to -45.6°C), and most preferably below -20°F (- 28.9°C). When the temperature is sufficiently low, the lupulin glands can be mechanically separated from the bract using a sieve or sifter. This separation is crucial to processing. When there is no material from the lupulin gland sticking to the hop cones or clogging the equipment, processing is most efficient and successful. The obtained powder is then packaged and used for brewing; however, compressing the powder into a pellet is preferable and makes the product easier to handle. This processing method is aimed at improving the quality and reduce degradation of α-acids and essential oils. The lupulin hop pellets produced this way are suited for use in brewing, in dry-hopping, and for use in hop beverages. With the increasing production of new high α-acid hops that are stickier lower processing temperatures may be required, and this may cause problems. The extremely low temperatures needed to reduce the stickiness could cause the hop cone to crumble resulting in unwanted materials in the fine fraction of lupulin. However, this can be remedied by using cryogenic separators to ensure that a pure product is produced (Vanevenhoven, Grogan and Zeigler, 2019).

2.7.5 Storage of Hops

Hops must be stored in cold conditions immediately after harvesting to preserve freshness and avoid spoilage. Depending on the desired final form, pellet, extract, etc., hops will usually be kiln-dried in the farm to moisture contents between 8 and 10% and baled. Baled hops will be used for

further processing. The main advantage of storing hops and hop products in suitable (cold) conditions is that the valuable components, the α-acids, are preserved very well at low temperatures (Biendl et al, 2015). To prevent excessive oxidation and polymerization of hop resin and essential oils hops are usually stored at temperatures between -2 and +4C in breweries. Depending on the hop variety even when stored at refrigeration temperatures deterioration of the important hop compounds can still occur. Studies have shown that hop pellets lose their bittering potential when they are stored at ambient temperatures, hence it is advisable that they be stored in refrigeration temperatures (Canbas, Erten and Ozsahin, 2001). Storage of hops at warm temperatures should be avoided, as that may cause chemical reactions and production of gasses that could cause the packing to rupture further increasing hops oxidation. Oxidation that occurs due to air exposure causes degradation of bittering acids and aroma compounds and consequently increases the hop storage index (HSI) of hops. The hop storage index is used to show if the hops are fresh or aged, HSI values below 0.31 indicate fresh hops (Bamforth, 2006). There are several factors that determine the quality of hops and it is not easy to single out one factor as the most vital to determine hop's quality. Besides the storage temperature, other factors in the hop quality chain such as, hop processing, harvest practices and processing, year of vintage and hop packaging can affect the overall quality of hops during storage (Srečec, Rezić, Šantek, and Marić, 2008).

2.8 Effects of Age on Hops

The content of valuable components in hops, mainly the α-acids and essential oils, will decrease over time. Some studies have reported that hop α-acid losses over time follow a first order kinetic equation, meaning that the rate of loss is proportional to the amount of α-acids present and the losses obey an exponential decay law (Green, 1978; Malowicki and Shellhammer, 2005). When comparing how age affected hops and liquid carbon dioxide extracts after being stored for a period of 11 months, it was found that in hops the α- and β-acids had degraded and this resulted in fewer iso-α-acids in the beers brewed with these hops. The loss of iso-α-acids was relative to the degradation products. However, in hop extracts the α-acids and β-acids remained constant as the extracts were more stable than hops during storage. Just as the α-acids and β-acids in hops the volatile compounds in the hops degraded as well. Those volatile compounds that were in the liquid carbon dioxide extracts remained stable during storage. (Priest, Boersman and Bronczyk, 1991).

As the α-acids content decreases during, the hop storage index (HSI) increases in value, meaning that the quality of hops decreases. The degree of degradation is dependent on hop variety. Studies have shown that certain varieties deteriorate much faster than other varieties at the same temperature and aging time. In their study after storing 9 hop varieties for 10 months at 72°F they found that the variety 56013 (Expt'I) was most unstable compared to the other varieties with an HSI value of 1.52. Early Cluster (clone E-2) was most stable with a value of 0.32 after 10 months of storage, Yakima Cluster (clone L-1) also showed stability with a value of 0.33 after 10 months (Likens, Nickerson and Zimmermann, 1970).

2.9 Degradation of α-and β-acids during storage

During storage α- and β-acids undergo a fast oxidation, and a recent study reported that hard resin fractions are believed to be mainly made of the oxidation products from α- and β-acids (Hao, Speers, Fan, Deng, and Dai, 2020). Factors such as temperature, availability of oxygen, time, and light exposure are responsible for the degradation of hop constituents especially the α-and β-acids. While the reduction in α-acids content is dependent on the variety of hops as previously mentioned (Mikyška and Krofta, 2012), it is usually slowed down by the use of low temperature during storage and shipping in combination with multi-laminated foil packaging material that acts as a barrier against light and oxygen (Tedone et al, 2020). The brewing value of hop pellets is reduced when hops are stored at high temperatures. The reduction in α-acid content is partly due to its conversion into iso-α-acids. Studies have demonstrated that when kept in hermetically sealed bags at temperatures between -10°C and -20°C hop pellets did not lose their bittering potential for up to 12 months (Srečec, Rezić, Šantek and Marić, 2009; Canbaş and Özşahin, 2001). When exposed to high temperatures during drying α-acids content in cascade hops were found to drop significantly after a storage period of 17 months. When dried at 140°F (60°C) the α-acids content reduced by 65%, at a drying temperature of 170°F (77°C) the α-acids degraded by 71% after being stored for 17 months (Weber, Jangaard and Foster, 1979). On the other hand, β-acid content remains stable through storage; however, as it has been mentioned previously, β-acids are of little interest in brewing due to their low solubility in wort and low bittering power compared to the α-acids. β-acids are highly stable even when stored at temperatures higher than 20°C and under anaerobic conditions they remain stable, but under the same conditions α-acids are lost. In the

study on Saaz, Sladek, Premiant and Angus hops it was observed that under aerobic conditions at 20°C there was a significant loss of β-acids with a loss of up to 83% for sladek hops and 51% for Angus after 1 year of storage ((Mikyška and Krofta, 2012). Stability of bittering substances is dependent on the pH. In general, α-acids are more stable in acidic conditions than in basic conditions where they can be converted to iso-α-acids and be more prone to oxidation (Steenackers et al, 2015). The presence of some polyphenols in hops help to reduce the loss of α-acids, due to the possession of 3',4' – dihydroxyphenol which is capable of causing a lag phase in the enzymatic oxidation of α-acids as the phenolic compound is the one degraded by the α-acid oxidase enzyme instead of the α-acids (Williams and Menary 1988; Williams 1989).

2.10 Hop Color

During harvest season, the color of hop cones is allowed to change from a bright green color which shows immaturity then to yellowish green color to show maturity. But the maturation color change rate of hops is mostly cultivar and weather condition dependent (Biendl et al, 2015). A bright green color might have been preferred by brewers historically over the brownish color of hops. To avoid excessive heating from the die, temperature should be limited to 50-55^{o}C which can be done by changing the die or by using nitrogen or CO_2 gas to cool the exit (Roberts, 2016).

2.11 Current Industry Practices on Preventing Hop Oxidation

The best packing methods for hops is to be under vacuum using an oxygen barrier or multi-laminate barrier packaging and flushing with an inert gas. To protect the hop's valued qualities hops are stored away from air in cold temperature at 30^{o}F to -5^{o}F (-1^{o}C to -21^{o}C) as cold temperatures slow down their oxidation (Garetz, 2015). To avoid air from entering into laminated bags and causing oxidation of hop components, the bags and their seal must remain intact. When a bag is poorly sealed when being closed and/ or when there is a pinhole puncture to the bags, air will diffuse into the bags and cause oxidation. Thus, it is important to have quality control checks to ensure that the sealed bags are of good integrity (Roberts, 2016). When packing hops in specialty barrier bags, the hops are first weighed before being vacuumed. After this vacuuming the hops are carefully flushed with nitrogen gas, to remove any traces of air/ oxygen that can lead to oxidation. The bag is then vacuumed again to remove the nitrogen gas and then heat sealed. An impermeable

seal is made by the heat impulse bar during sealing. Since the nitrogen gas is removed, the pellets are packed as dense blocks with specific tracking information labeled on them. This helps with tracing the pellets back to the specific field they were grown in case there are any concerns with the hops. The hops are stored in cold storage until they are sold (Gorst Valley Hops, 2018).

CHAPTER 3. MATERIALS AND METHODS

3.1 Hop Samples

Hop pellets from two varieties, Cascade (aroma hops) and Chinook (bittering hops), were purchased from a farm in Knightstown, IN. Each lot consisted of 20 pounds of hop pellets from the 2019 growing season. Hop cones were harvested during summer 2019, kiln dried, and packed in bales and stored at 4^{o}C until December 2019. In December, hop cone bales were pelletized and stored in heat sealed and nitrogen flushed 10-lb size laminated foil bags.

3.2 Sample storage

For each hop variety, the two 10-lb bags of pellets were combined in a large bag and thoroughly mixed. Then, 75 g of hops were repackaged into 15.3 cm x 13.3 cm aluminum foil lined bags. Approximately 120 small bags were obtained for each hop variety. After repacking, half of the bags (nitrogen-exposed samples) were flushed with nitrogen gas in the headspace while the other half was not flushed (air-exposed samples), before being heat sealed. The bags were flushed and sealed using a PVG Industrial Vacuum Sealer (Model PV-GA-36 Packaging Aids, PAC Machinery, San Rafael, CA) following the protocol used by the hop producer. Twenty bags from each treatment (nitrogen- or air- exposed) were randomly selected and stored at one of each of the following temperatures: 4°C, 25°C or 35°C for up to 160 days. Sampling times were assigned as shown in Table 3.1. At each sampling period, one bag from each gas-type and each temperature was retrieved. The 75 g of pellets were ground to a fine powder using a coffee grinder and samples were analyzed as described below. The remaining powder was vacuum packaged and stored at -18^{o}C until completion of the study.

Table 3.1 Sequence of sample testing per hop variety. Boxes marked with an X indicate when samples were retrieved for analysis

Temperature	**4°C**		**25°C**		**35°C**	
Time (days)	**Air**	**Nitrogen**	**Air**	**Nitrogen**	**Air**	**Nitrogen**
0	X	X	X	X	X	X
2	N/A	N/A	N/A	N/A	X	X
4	N/A	N/A	N/A	N/A	X	X
6	N/A	N/A	N/A	N/A	X	X
7	X	X	X	X	X	X
14	X	X	X	X	X	X
21	X	X	X	X	X	X
28	X	X	X	X	X	X
35	X	X	X	X	X	X
42	X	X	X	X	X	X
56	X	X	X	X	N/A	N/A
70	N/A	N/A	N/A	N/A	X	X
84	X	X	X	X	N/A	N/A
112	X	X	X	X	N/A	N/A
140	X	X	X	X	N/A	N/A
168	X	X	X	X	N/A	N/A

3.3 Total α- acids and, total β-acids content and Hop Storage Index in hop pellets quantified by UV/Vis Spectrophotometry

Determination of α- and β-acids content of hops was done using a UV/ Visible spectrophotometer (DU 800, Beckman Coulter, Brea, CA) according to the method Hops – 6A as defined by the American Society of Brewing Chemists (ASBC). Briefly, 2.5000g ± 0.001g of finely ground hops was placed in a 125 mL extraction bottle to which 100 mL of toluene was added. The bottle was stoppered tightly and then placed in a mechanical shaker for 30 minutes. From there the contents were centrifuged at 2000 rpm (693g) for 13 minutes. This solution contained an equivalence of 50 mg of hops per mL. Next, 2.5 mL of the toluene extract was diluted with 47.5 mL of methanol (Dilution A). An appropriate amount of dilution A was diluted with alkaline methanol (Dilution B) so that absorbances at 325 and 355 nm fall within the most accurate range of the instrument used. In this case, samples were diluted to obtain absorbance values lower than 1.0. The absorbance of dilution B was determined at 355, 325 and 275 nm after setting the instrument to zero absorbance using a blank prepared with 2.5 mL of toluene and following the same dilution sequence of the sample. Absorbance readings were taken rapidly after dilution B was made to avoid decomposition of constituents by UV light. Spectrophotometric method Hops – 12 of ASBC was used to determine the hop storage index (HSI) which provides an indication of hop freshness. The following equations (Eq. 1-4) were used to calculate the dilution factor (d), total α-acid and total β-acid content (%), and the hop storage index (HSI):

$$Dilution\ factor\ (d) = \frac{\text{vol.dil A (mL)} x\ vol.\ dil\ B(mL)}{\text{500 x aliq. extract (mL) x aliq. dil A (mL)}} \quad \text{(Eq. 1)}$$

$$\alpha - \text{Acids}, \% = d\ (-51.56A355 + 73.79A325 - 19.07A275) \quad \text{(Eq. 2)}$$

$$\beta - \text{acids}, \% = d\ (55.57A355 - 47.59A325 + 5.10A275) \quad \text{(Eq. 3)}$$

$$Hop\ storage\ index\ (HSI) = \frac{\text{A275}}{\text{A325}} \quad \text{(Eq. 4)}$$

3.4 Total α- acids and Total β-acids, humulones, and lupulones content in hop pellets quantified by HPLC-DAD

Determination of total α- acids, and total β- acids cohumulone, n + adhumulone, colupulone and n + adlupulone content in hop pellets by HPLC was done according to method Hops – 14 by ASBC. A 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with diode array

detector (DAD) was used for this method. Sample separation was performed using a 250 × 4 mm, 5-µm ODS RP18 Nucleosil C-18 column (Machery-Nagel, MA). Briefly, for hop pellets, approximately 15g of hop pellets were ground to a fine powder using a coffee grinder. Then, 10g of the finely ground sample was weighed into a 250 mL extraction bottle to which 20 mL of methanol and 100 mL of diethyl ether was added. The bottle was tightly stoppered and shaken using an orbital shaker for 30 minutes, after which the bottle was carefully opened and 40 mL of 0.1M hydrochloric acid solution was added. The bottle was re-stoppered and shaken again for at least 10 minutes. The bottle was allowed to stand for 10 minutes to allow for separation of the organic and aqueous layers. Five mL of the supernatant ether phase was pipetted into a 50 mL volumetric flask and made up to volume with methanol. The contents of the flask were mixed carefully. The methanol dilution was filtered through a 0.45 µm nylon filter and filled into glass vials. A volume of 10 µL was injected each time. Detection was done at a wavelength of 314 nm. All sample extractions were performed in duplicate and injected into the HPLC system.

Quantification of cohumulone, n-humulone, and adhumulone, as well as of colupulone, n-lupulone, and adlupulone was obtained from the area of the peaks in comparison to peak areas generated from an international calibration extract obtained from ASBC (St. Paul, MN). Specifications of the calibration extract were reported as: total α-acids 42.58%, cohumulone 10.98%, n + adhumulone 31.60%, total β-acids 26.54%, colupulone 13.02%, and n + adlupulone 13.52%. The calibration standard was diluted as indicated in the method and injected four times. Four individual response factors were calculated for the cohumulone, *n*- + ad-humulone, colupulone, and *n*- + ad-lupulone. The four calculated response factors were averaged and used for quantification of individual components in the samples. The response factor and component percentages were calculated using equations (Eq. 5-6) as follows:

$$Response\ factor\ (RF) = \frac{\text{mass of calib.ext.(g)x conc.of component in calib.ext.(\%)}}{\text{area of component in calib. ext.}} \quad \text{(Eq. 5)}$$

$$Component, \% = \frac{DF\ x\ average\ sample\ peak\ area\ of\ component\ x\ RF}{\text{mass of sample (g)}} \quad \text{(Eq. 6)}$$

Where DF is dilution factor, and DF = 2 for hop pellets.

3.5 Hop Color Measurement

Color of ground hops was measured using a LabScan XE Spectrophotometer (HunterLab, Reston, VA), with 0°/45° optical geometry, illuminant D65 and 10° observer, which was connected to a personal computer. The colorimeter was standardized using a black and a white standardizing tile that were provided with the instrument (L= 94.02. a= -1.42, b= 1.55, for the white tile). Color measurements were based on three color co-ordinates using the CIELab scale, L*, a* and b*. L* indicated lightness (0 – 100) with 0 being black and 100 being white**.** The coordinate a* when positive (+) indicated redness while (-) is for greenness, and b* coordinate (+) indicated yellowness and (-) blueness. An amount of 5g of ground hops was placed in a glass sampling cup which was placed over the aperture and covered for readings to be taken. Measurement were taken in triplicate and average values were used for calculations. The obtained CIELab scale color parameters were used to calculate chroma (C*), hue (h) angle and the total color difference (ΔE) using equations (Eq. 7-9) as follows:

$$Chroma\ (C^*) = \sqrt{a^{*\,2} + b^{*\,2}} \qquad \text{(Eq. 7)}$$

$$Hue\ angle\ (h^\circ) = \left(\frac{ATAN\left(\frac{b^*}{a^*}\right)}{6.2832}\right)360 \qquad \text{(Eq. 8)}$$

$$\Delta E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2} \quad \text{(Eq. 9)}$$

Where L*, a*, b* are the color parameters at any given time, and L_o^*, a_o^*, and b_o^* are the color parameters obtained for each variety at the beginning of the study (time = 0 days)

3.6 Total Oil Content by Steam Distillation

The oil content of each hop variety was determined by an external laboratory (AAR labs, Madison, WI) according to ASBC method Hops – 13 using steam distillation. Briefly, 100g of hop pellets were mixed in with 3000 mL of deionized water in a round bottom boiling flask. The distilling calibrated receiver was filled with water through the top of the condenser before start of distillation. The water was brought to a rolling boil and the distillation rate was regulated such that 25 – 35 drops fell from the tip of the condenser per minute. The distillation process took 4 – 7 hours and the observed hop oil volume collected in the trap was measured when distillation was complete. The amount of essential oil (mL/100g) was calculated using the following the following equation (Eq. 10):

$$Total\ essential\ oils, mL/100g = \frac{\text{V x 100}}{\text{W}} \qquad \text{(Eq. 10)}$$

Where V = mL in oil receiver and W = weight of hop sample.

3.7 Moisture by air oven method

Moisture content was determined according to ASBC method Hops – 4C by an external laboratory (AAR labs, Madison, WI). Briefly, 2.5g of each unground hop pellets sample was placed in a covered, dried, and weighed moisture dish. The hops and dish were weighed to 0.001g and placed in the oven. The dish cover was removed, and the hops dried for 1 hour at 103°C - 104°C. After the hour had elapsed the cover was replaced on the dish while the dish was still in the oven. The dish was removed to the desiccator and allowed to cool, the dish and dried sample were weighed to 0.001g.

To calculate the moisture content in wet basis the following equation was used:

$$Moisture\ in\ hops, \% = \frac{loss\ in\ wt\ x\ 100}{wt\ of\ sample} \qquad \text{(Eq. 11)}$$

3.8 Kinetic modeling

Data collected from the storage study analyzed by HPLC, i.e. total α-acids, cohumulone, n + adhumulone, total β-acids, colupulone, n + adlupulone, were plotted against storage time and analyzed for best fit to determine the order of the reaction that best described the experimental data. Zero, first, and second order reactions were tested by plotting either the concentration (C), the natural logarithm of concentration (lnC), or the inverse of concentration (1/C) as a function of time. Linear regression of the plots were determined and the order selected based on those lines that resulted in the highest coefficient of determination (R^2). Most results conformed to first-order degradation kinetics, as reported by Green (1978) for degradation of bittering acids, and could be described by Eq. (12) as

$$\ln C = \ln C_o - kt \qquad \text{(Eq. 12)}$$

Where C is the concentration of total α- or β-acid or of individual components at time t (days), C_o is the concentration at the initial time ($t_o = 0$), and k is the degradation rate constant. Degradation

rate constants (k) were determined from the slopes of the regression lines obtained when the natural logarithm of C (lnC) was plotted as a function of storage time, t.

An Arrhenius type equation was used to determine the dependence of the degradation rate constant on temperature. However, for all components, except for colupulone the relationship was valid only for the range within 25 to 35^{o}C, since negligible degradation was observed for samples stored at 4^{o}C. The activation energy was obtained using the following equation (Eq. 13):

$$\ln(k) = \ln(k_{ref}) - \left(\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \qquad \text{(Eq. 13)}$$

Where k is the degradation constant at a temperature T, and k_{ref} is the degradation constant at a reference temperature, T_{ref}, E_a is the activation energy, and R is the universal gas constant.

3.9 Statistical analysis

All sample extractions and other measurements were performed in duplicate. Results are presented as the mean ± standard error. A generalized linear model was used to analyze the data using statistical software Minitab 19. Differences within groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's test. The significance of differences was defined at the $P < 0.05$ level. Values represent average of triplicate (for spectrophotometric method and color analysis) and duplicate (for HPLC-DAD) analysis ± standard deviation.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 Effects of Storage Temperature on hop α-acids Content

Storage temperature was the most important factor affecting the α-acid content of hops. Increasing the storage temperature to 25 or 35ºC caused a decrease in the α-acids content for both hop varieties as shown in Fig. 4.1 for Cascade total α-acids, and α-acids components in Fig 4.2, and in Fig. 4.3-4.4 for Chinook hop pellets and α-acid components. The α- acids are the most important bittering acids and are stored in the lupulin glands of the hop flower; by themselves, α-acids are mostly insoluble in water and do not impart much bitterness unless converted to iso-α-acids during the boiling step in brewing (Cattoor et al, 2013).

As the storage temperature increased the loss of α-acids was more evident in the pellets. Cascade hop pellets stored at 4°C did not exhibit a significant decrease in α-acid content during storage for up to 168 days. The average content of α-acids for Cascade pellets stored at 4ºC fluctuated around 5.38% for samples stored under air and 5.47% for samples stored under nitrogen; however, the effect of gas type was not statistically significant ($p>0.05$). When storage temperature increased to 25°C Cascade hop pellets showed α-acid degradation values of 18.5% for nitrogen-flushed and 18.7% for air-exposed samples as determined by the spectrophotometric method; whereas degradation values of 14.5% for nitrogen-flushed, and 14.5% air-exposed were calculated from the HPLC method. The observed differences are due to quantification method, and not due to the type of gas in the headspace. The spectrophotometric method requires the absorbance value at three different wavelengths to calculate the total α-acid content using a regression equation, whereas the HPLC method is based on the chromatographic separation of individual components whereby retention time and peak areas of eluted components are compared to the retention time and peak areas of a standard sample of known composition obtained from ASBC. Therefore, similar to the results at 4ºC, the effect of gas type in the headspace was not statistically significant as shown in Fig. 4.1. Results from Tukey's test for means comparisons indicated that at 4ºC, the total α-acid content was not significantly different at various storage times, except for the sample stored for 42 days under air, which showed a slightly higher value (5.8%). However, it is possible that the observed higher value would be the result of natural variation in that sample. On the other

hand, comparison of means for total α-acids in Cascade samples stored at 25°C indicated that the α-acid content became significantly different from the initial concentration after 56 days for air-exposed samples, and after 84 days for nitrogen-flushed packages. According to these results, it could seem like nitrogen might have a protecting effect, retarding degradation reactions; however, at the end of the study, after 140 days, there were no significant differences between the total α-acids content of nitrogen-flushed (4.7% α-acids) or air-exposed (4.7%) samples.

In other studies (Skinner, Kavanagh and Clarke, 1979; Wain, Baker and Laws, 1977) it was observed that the storage of hops under anaerobic conditions helped to decrease α-acid degradation although it did not completely stop it. They suggested that oxidizing agents responsible for the decrease in α-acids content as well as their precursors were already present in the product and were responsible for the residual oxidation of the acids even in the absence of oxygen.
The extent of degradation observed at 25°C in the present study was much lower than that reported by Forster (2002) and was also lower than a study reported in Kunze (2014) that showed up to 25% degradation in total α-acids after 2 months of storage at 18°C. The loss of α-acids under inert conditions is due to conversion of the α-acids to iso-α-acids (Forster, 2002). Though there was a loss in α-acids at 25°C, those samples could still have brewing value and brewers would compensate for losses in α-acids by adding larger amounts of hops. In order to determine whether stored hops are still usable, the hop storage index (HSI) needs to be quantified as discussed in a later section.

When storage temperature was further increased to 35°C there was a drastic loss of α-acids for the pellets under both atmospheric conditions as time increased. Analysis of means indicated that α-acid content became significantly different in samples stored for 14 days irrespective of the type of gas. The amount of α-acids lost for air and nitrogen-exposed samples was 71.7% and 63.0% respectively for samples analyzed by spectrophotometric method, whereas samples analyzed by HPLC showed losses of 54.5% (air) and 52.7% (nitrogen) in Cascade hop pellets after 70 days of storage. Similar to results at 25°C, hop pellets stored at 35°C temperature showed a difference in losses observed under different gas in headspace. Nevertheless, at 35°C, the difference between gas types, although small, was statistically significant. After 70 days, the final concentration of α-

acids in nitrogen-exposed samples (2.6%) was slightly higher than that of air exposed samples (2.2%) as shown in Fig. 4.1. These results show that the type of headspace gas under which hops pellets are packaged matters especially at high temperature. The study at 35^{o}C was terminated after 70 days since sample bags swelled and the color and aroma of those hop pellets were uncharacteristic even of aged hops and the bags were swollen, indicating spoilage.

Degradation of α- acids in Chinook hop pellets followed the same trend as that in Cascade pellets. At 4°C there was no significant difference exhibited by the α-acid content during 168 days of storage. At 4°C the α-acid content was about 6.3% for the whole duration of the study for samples stored under nitrogen flush and those exposed to air as shown by Fig 4.3. When the temperature was increased to 25°C and 35°C there was a more noticeable change in the α-acid content of the hop pellets. At 25°C observed α-acids losses were 19% for air exposed and 23.4% for nitrogen flushed samples as determined by spectrophotometric method; whereas losses of 15.4% for air-exposed and 13.1% for nitrogen flushed samples were determined from HPLC method. Similar to the Cascade pellets there was no significant difference with regards to gas type in the headspace. The results from Tukey's test for means' comparison showed that there was no significant difference at different storage times at 4°C for Chinook pellets. At 25°C the Chinook pellets in storage had a significant difference in total α-acids. The α-acid concentration became significantly different from the initial content from day 35 for the air-exposed samples and day 56 for nitrogen flushed samples. During the period of the study, 140 days, the results show that nitrogen had a very slight protective effect on the α-acids of Chinook hops as at the end of the study the content of the of nitrogen flushed pellets was 5.5% while the content of the air exposed samples had a content of 5.3%.

Chinook hop pellet samples stored at 35°C experienced a drastic loss in α-acids. There was an α-acid degradation value of 95.2% and 84.4% for nitrogen flushed and air exposed samples respectively as determined by the spectrophotometric method. However, loss of 87.7% and 63.9% for nitrogen flushed and air-exposed samples respectively, were determined from HPLC method. The storage of Chinook samples at 35°C was also terminated after 70 days as there was swelling of bags which indicated spoilage. Comparison of means for total α-acids at 35°C indicated that

there was a significant difference from the initial α-acid contents from day 14 regardless of the headspace gas in the sample bags. There seemed to be a reverse effect on headspace gases for Chinook compared to Cascade samples. For Chinook pellets the nitrogen flushed samples had a lower final α-acid content (0.8%) compared to air-exposed samples (2.2%), shown in Fig 4.3. Though the varieties followed the same trend in their loss of α-acids they did not degrade at the same rate. Degradation in Cascade pellets was much slower than in pellets from Chinook.

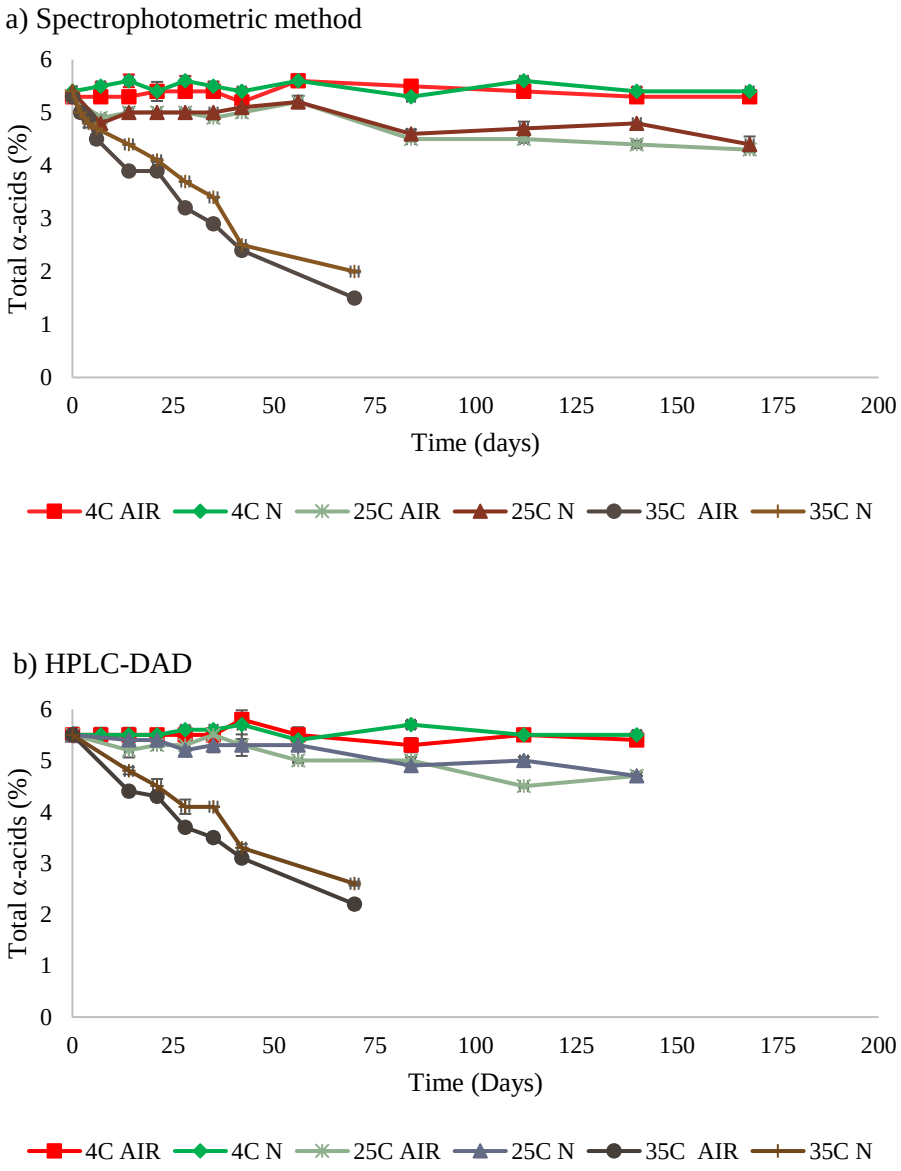


Figure 4.1 Total α-acid content (%) of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric methods or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation.

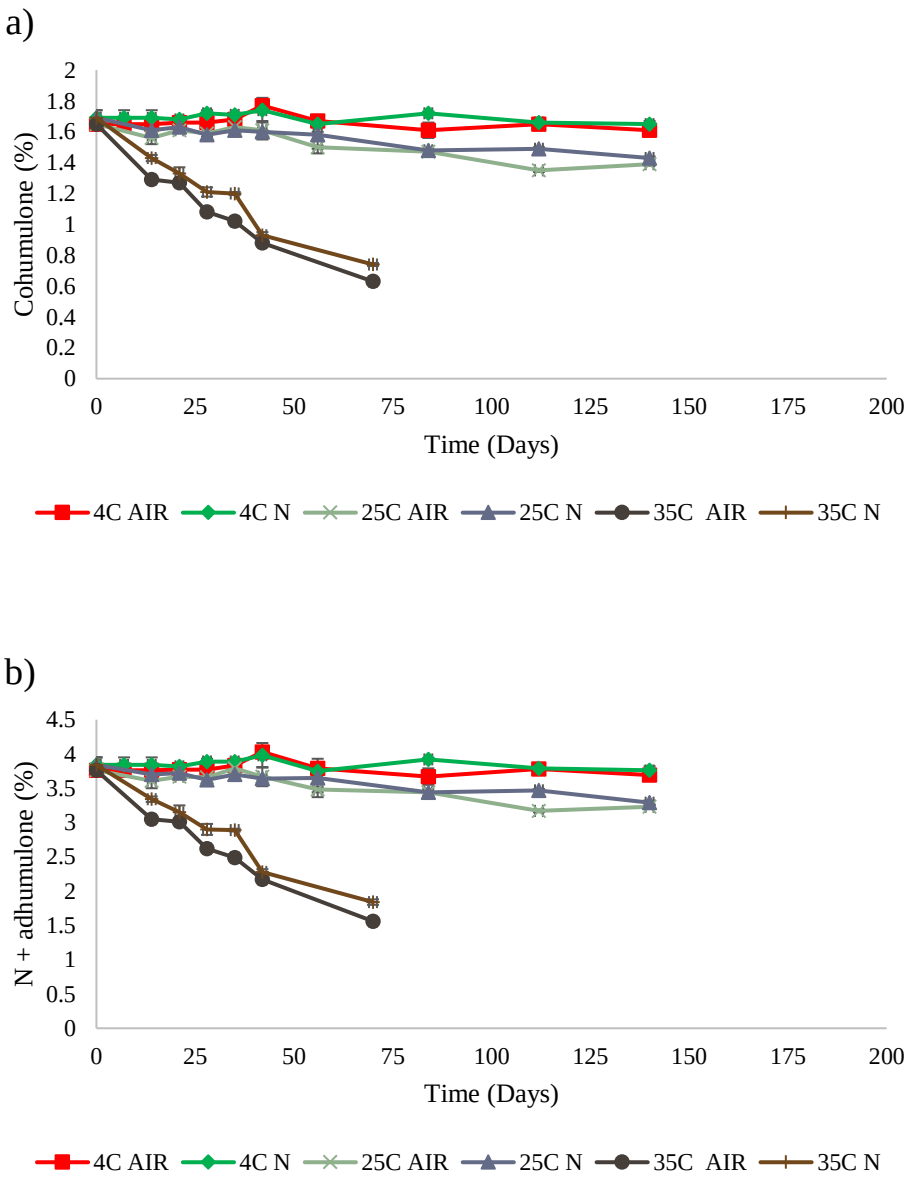
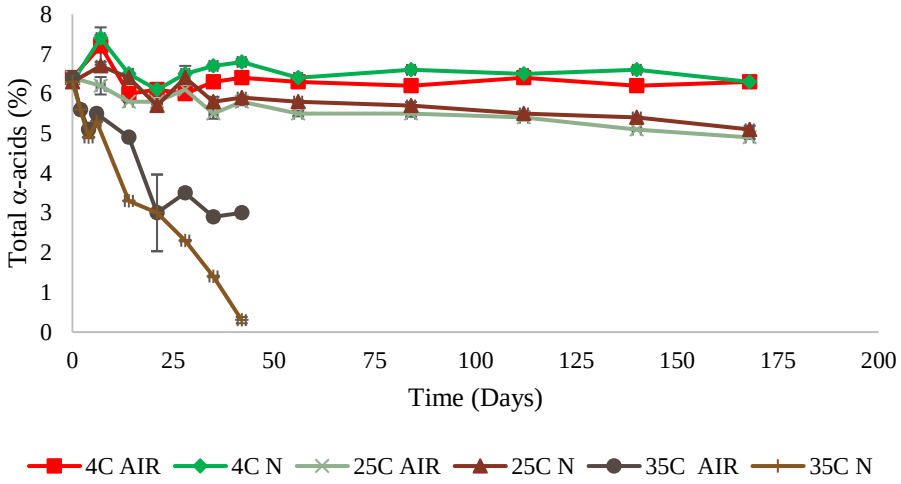


Figure 4.2 Content (%) of a) Cohumulone and b) N + Adhumulone of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation.

a) Spectrophotometric method



b)HPLC-DAD

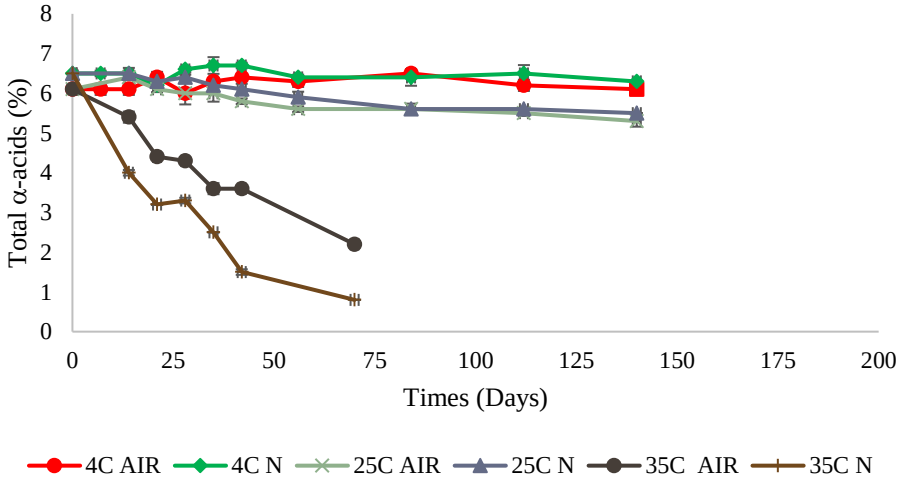


Figure 4.3 Total α-acid content (%) of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric method or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation.

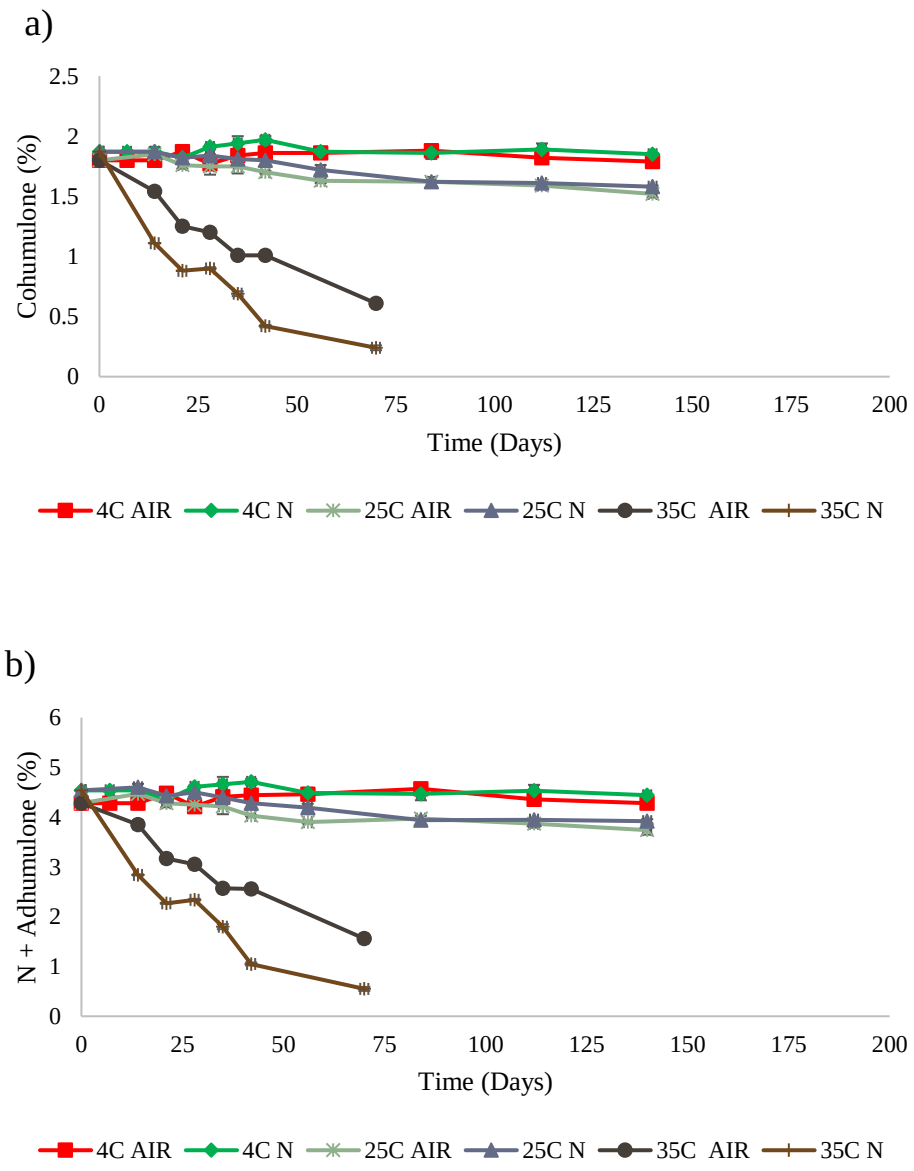


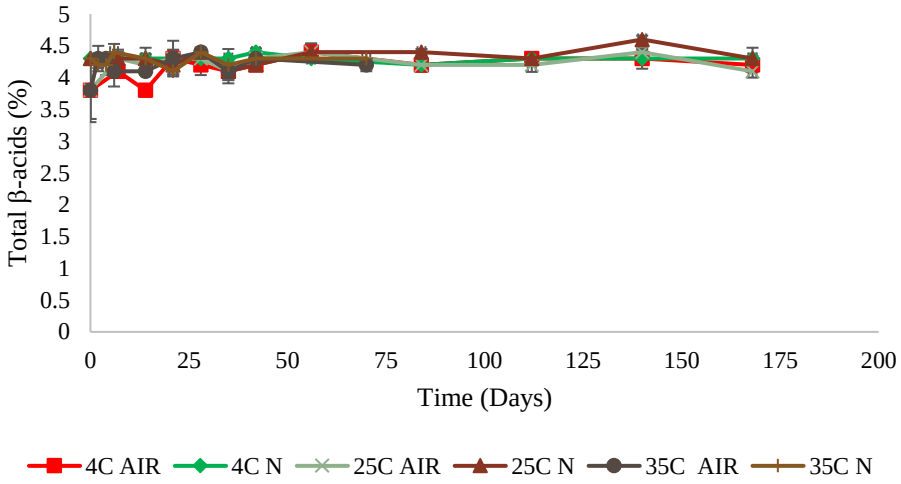
Figure 4.4 Content (%) of a) Cohumulone and b) N + Adhumulone of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation.

Hop α-acids comprise mainly cohumulone, N humulone and adhumulone. Most brewers prefer to have varieties with small cohumulone levels, and according to the HPLC results both Cascade and Chinook varieties have less cohumulone content compared to N +adhumulone. At 4°C the comparison means did not show any significant difference in all these components in both the hop varieties. When comparing the means at 25°C for Cascade samples exposed to air there was a significant difference from the initial values at day 56 for both cohumulone and N + adhumulone. For nitrogen flushed samples there was a significant difference from day 28 for cohumulone while there was no significant difference for N + adhumulone. For samples that were stored at 35°C comparison means showed that there was a significant difference from day 28 regardless of the headspace gas in the samples. Chinook samples at 4°C did not show a significant difference, as observed in Fig 4.4. At 25°C samples exposed to air showed a significant difference from day 42 for both cohumulone and N + adhumulone. For those samples that were nitrogen flushed cohumulone showed a significant difference from day 84 while N + adhumulone showed a significant difference from Day 56. Cohumulone and N + adhumulone showed a significant difference from day 35 for both air exposed and nitrogen flushed Chinook samples at 35°C.

4.2 Effects of Temperature on β-acids

β-acids in hop pellets were more stable compared to α-acids during storage. In inert conditions β-acids remain stable but when they are exposed to high temperatures (up to 30°C) for long durations they will degrade although not to the same extent as α-acids (Forster 2002). Mikyska and Krofta (2012), observed that in anaerobic conditions β-acids are stable during storage but when kept in open bags and exposed to air the loss is recognizable especially after 6 months of storage. In our study, β-acids remained stable throughout the whole duration of the experiment. The β-acid content for Cascade at 4°C fluctuated around 4.3% for samples analyzed by the spectrophotometric method, while the content fluctuated around 3.9% for samples analyzed by HPLC. This same behavior was observed for samples with both types of gas in the headspace. The comparison of means did not show any significant differences of total β-acids at 4°C over time. At 25°C and 35°C there was no significant difference for the β-acids in the Cascade samples, as shown in Fig 4.5-4.6. For Chinook samples at 4°C, 25°C and 35°C there was no significant difference for Chinook pellets at both atmospheric conditions, as shown in Fig 4.7-4.8.

a) Spectrophotometric method



b) HPLC-DAD

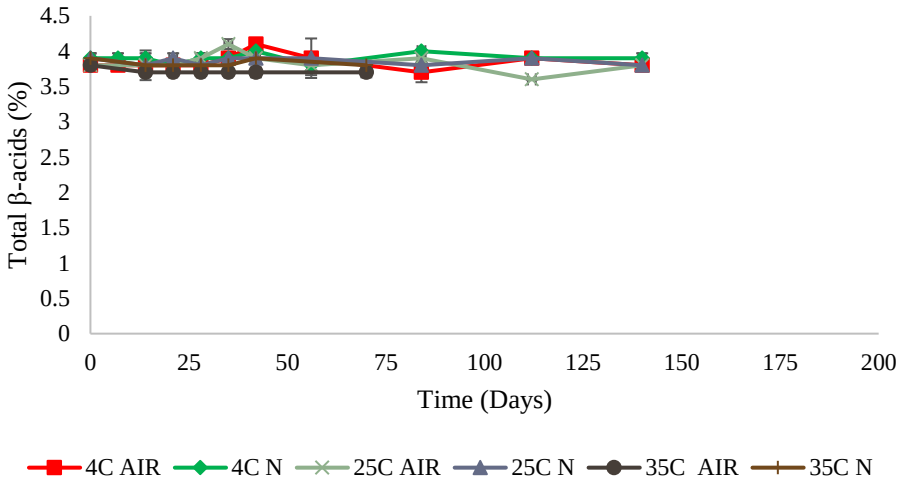


Figure 4.5 Total β-acid content (%) of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric methods or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation.

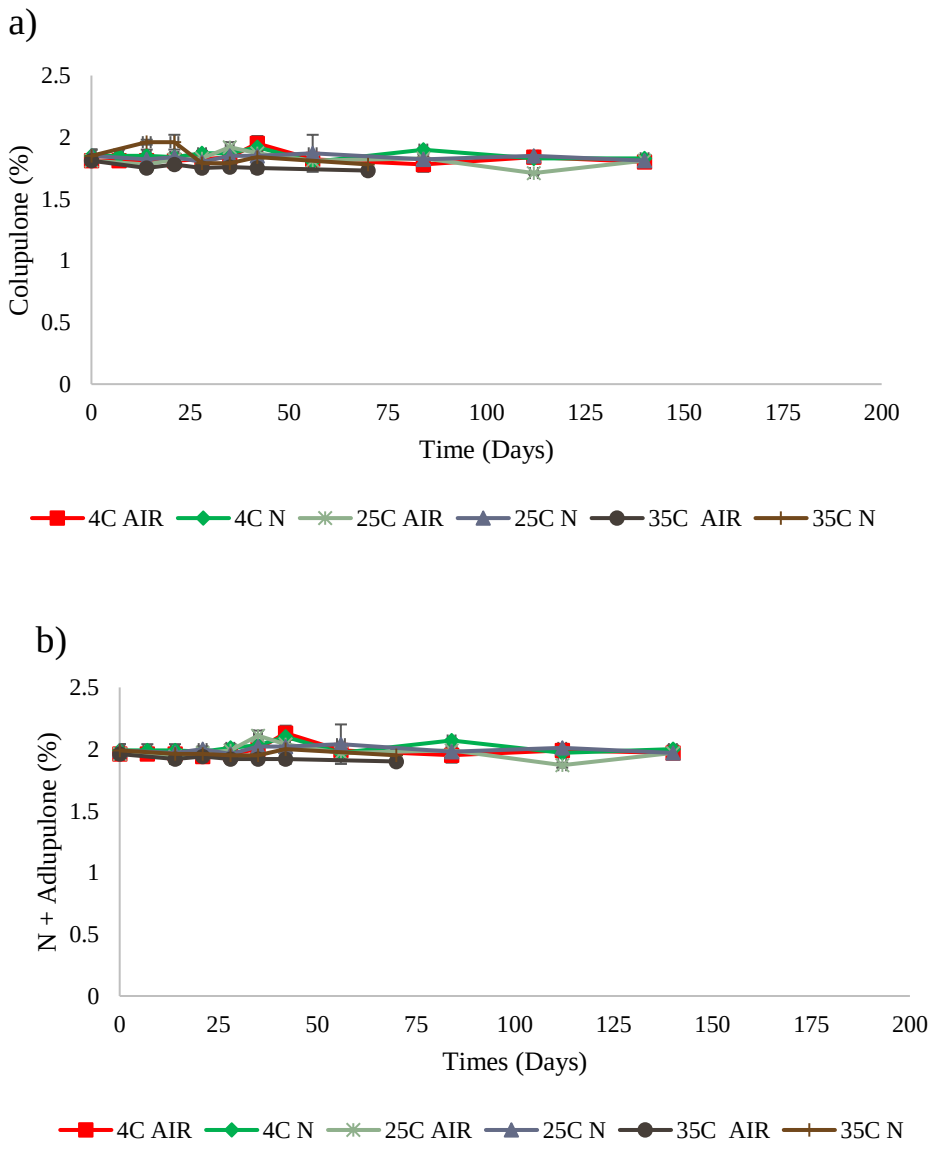


Figure 4.6 Content (%) of a) Colupulone and b) N + Adlupulone of Cascade hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation.

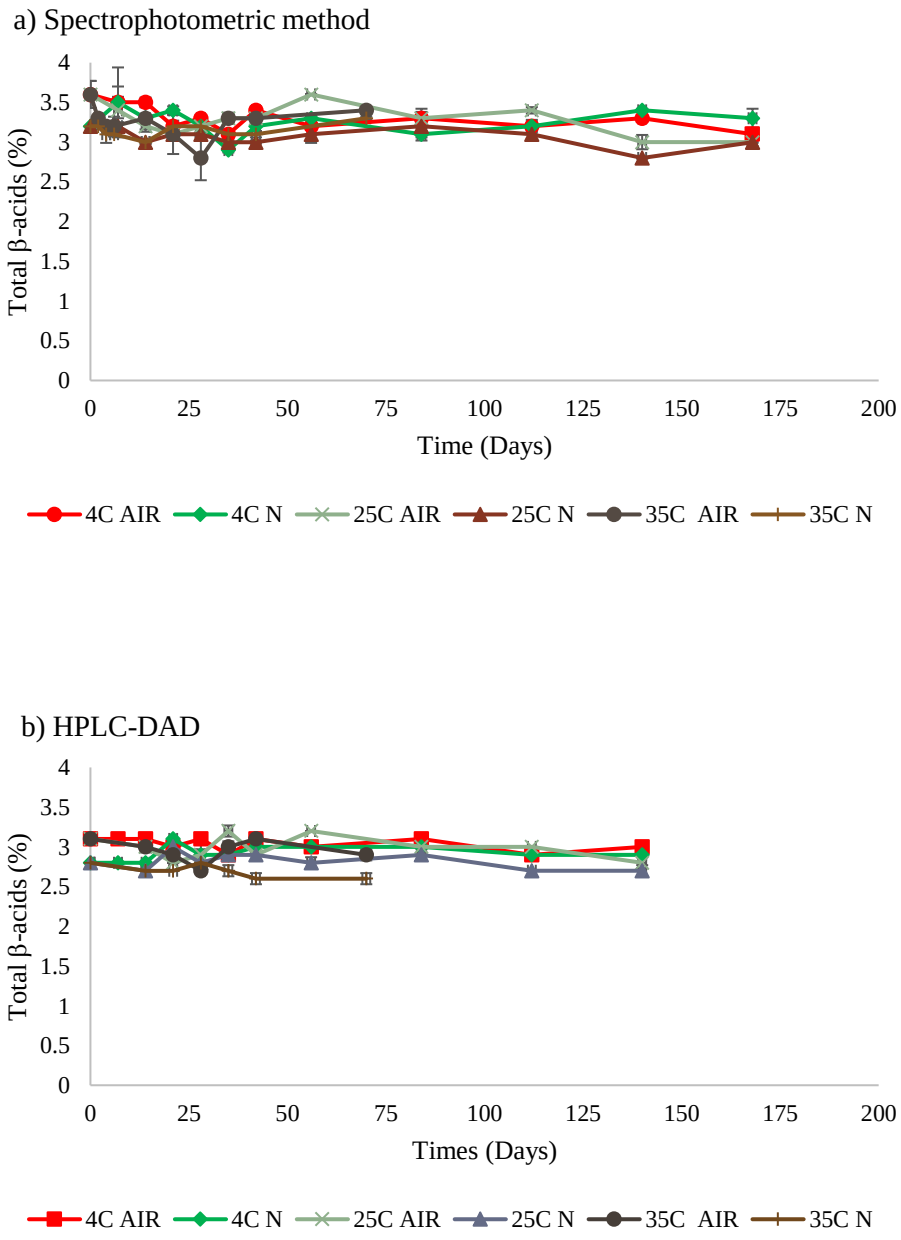


Figure 4.7 Total β-acid content (%) of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by a) spectrophotometric method or b) HPLC-DAD. Values represent average of triplicate (for spectrophotometric method) and duplicate (for HPLC-DAD) analysis ± standard deviation.

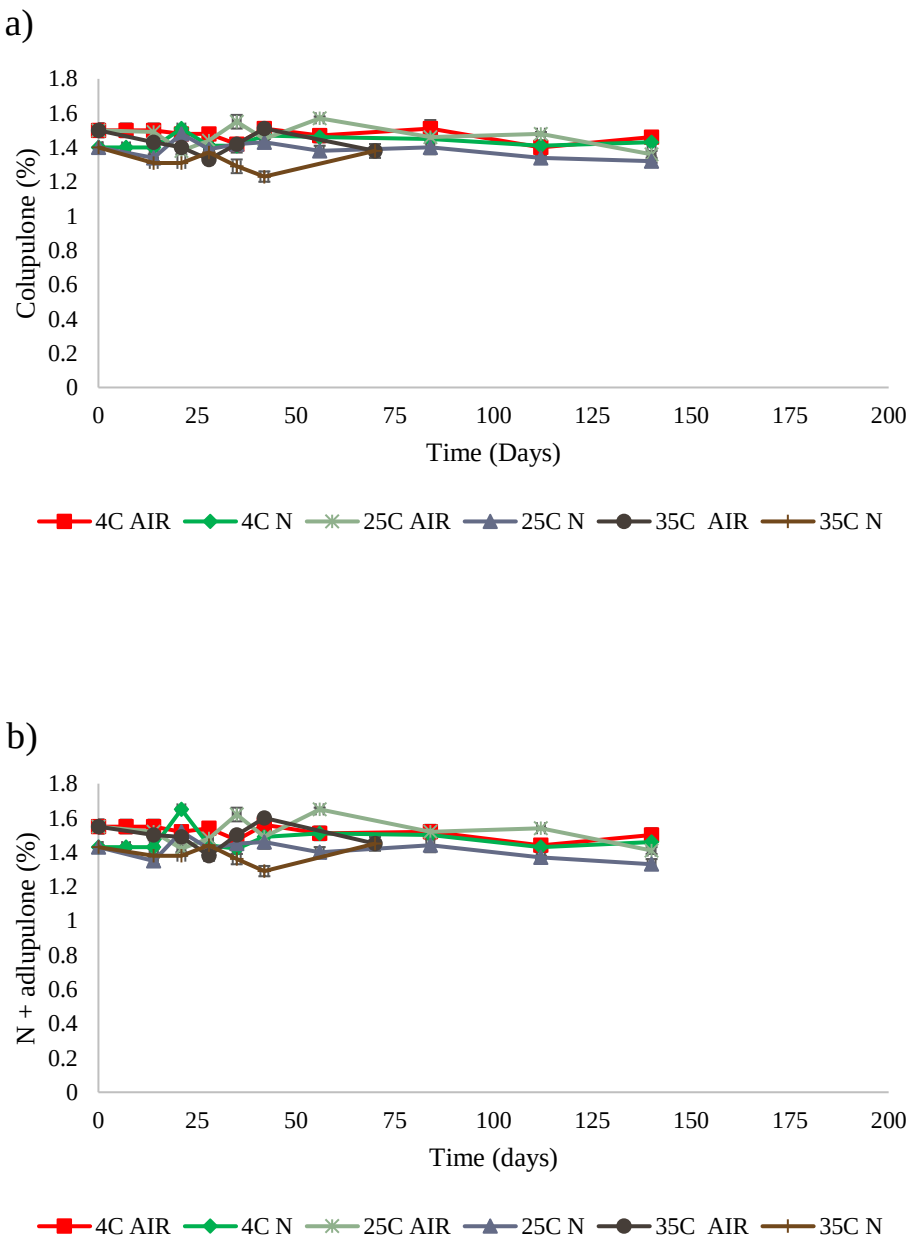


Figure 4.8 Content (%) of a) Colupulone and b) N + Adlupulone of Chinook hop pellets stored in air (AIR) or nitrogen (N) flushed packages at 4°C, 25°C or 35°C for up to 168 days as quantified by HPLC-DAD. Values represent average of duplicate analysis ± standard deviation.

4.3 Effects of Temperature on Hop Storage Index

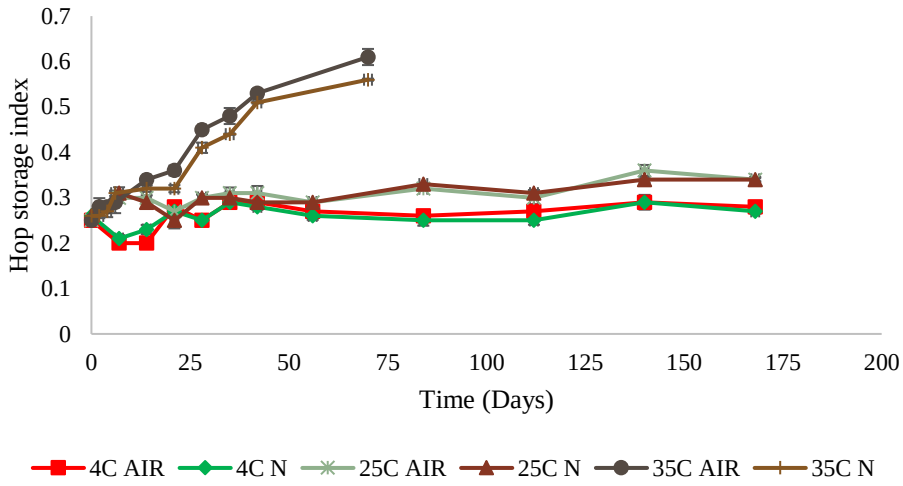
Hop storage index is effective in screening hop varieties to determine their storage stability (Likens, Nickerson and Zimmermann, 1970). Hop storage index (HSI) partially indicates how well hops were handled by growers during harvest and transportation. However, the losses of α- and β-acids in hops also depends on the variety, and on the α- to β-acid ratio in the hops. Oxidation is of particular importance in high α-acid varieties where the α/β ratio can be as high as 3:1. According to Nickerson and Likens (1979), when oxidation of high α-acid varieties occurs, the quantity of bitter products formed by oxidation of β-acids is not sufficiently high to compensate for the loss of bittering potential due to the loss of α-acids, thus the overall bittering potential of the hops decreases. However, in low α-acid varieties where the α/β ratio is close to 1:1, the decrease in bitterness due to loss of α-acids is compensated by the formation of oxidation products from β-acids and the overall bittering potential of low α-acid varieties remains practically unchanged. In general, the lower the HSI the more brewing value the hops have. HSI is the ratio of absorbance at 275 nm to absorbance at 325 nm of a toluene extract obtained from dried hop material in an alkaline methanol solution. The theory behind HSI calculation is that spectra of naturally formed oxygenation products of α- and β-acids from hops have a maximal absorption at wavelengths between 250 nm and 280 nm, whereas a mixture of α- and β-acids dissolved in alkaline methanol has a minimal absorption at 275 nm and maximal absorption at 325 nm and 350 nm (Nickerson & Likens, 1979; Tedone et al., 2020). Thus, the less degraded the α- and β-acids become, the lower the HSI value will be. Fresh hops have HSI values around 0.25. Large breweries that normally contract out their hops require that the HSI of supplied hops be between 0.3 and 0.4 to ensure the freshness of the hops (Nickerson & Likens, 1979). Srečec et al. (2008) showed that hop pellets with an HSI value lower than up to 0.50 are considered to be good for brewing as they did not have a negative impact on the sensory characteristics of beer. This beer was prepared by infusion mashing, boiled with hop pellets and after cooling the beer was fermented by lager yeast. Primary fermentation was done at 13°C for five days and the secondary fermentation at 2°C for 21 days. However, hops with an HSI value greater than 0.5 are expected to be more oxidized but could still be used for brewing as long as the hopping rates are adjusted. Fig. 4.9 shows values of HSI as a function of time and storage conditions for cascade and chinook hop pellets evaluated in our study.

Storage of hop pellets at 4°C resulted in acceptable HSI values that remained well below 0.4 for both hop varieties under both atmospheric conditions. At 4°C the hop storage index of the pellets ranged from 0.25 to 0.28 for Cascade for both atmospheric conditions, while for Chinook the range was from 0.24 to 0.26 after 168 days of storage. As storage temperature increased the HSI increased. This trend was observed in both hop varieties. At 25°C the HSI after 168 days of storage was still below 0.4 (final values ranged from 0.34 – 0.37), although they were slightly higher than those at 4°C. The effect of gas in the headspace was not significant within varieties. Thus**,** even after 168 days storage at room temperature, the hops were still of good brewing values if stored at 4 or 25^{o}C regardless of type of gas in the package. There was a difference at the highest storage temperature of 35^{o}C. The HSI increased continuously with storage time and in all cases became greater than 0.5 after just 70 days. At 35^{o}C the effect of variety became significant. Cascade had high HSI values but they were lower than the values observed for Chinook pellets as shown in Fig. 4.9. Some of the values were greater than 1.0 indicating extreme degradation. Results show that Chinook variety is more prone to aging than Cascade is. After 70 days at 35°C the highest HSI values recorded for Cascade and Chinook pellets were 0.61 and 1.27 respectively. There was also an observed difference in HSI of pellets stored under nitrogen and those exposed to air in one of the varieties. For Chinook, pellets stored under nitrogen had an HSI of 1.27 compared to 0.82 observed for samples stored under air after 70 days. Experimental results show that the higher the α-acid loss, the higher the HSI. Figure 4.3 shows that at 35°C Chinook pellets lost the most α-acids and in Figure 4.9 it is seen that at the same temperature the HSI is highest. However, the reason why nitrogen-stored samples showed higher HSI values than air-exposed ones is not clear, though this could have been due sample variation. Cascade pellets, on the other hand, showed a similar trend with respect to increasing HSI at longer times, but the effect of gas-type in the headspace was not significant. Likens et al. (1970) and Nickerson & Likens (1979) observed a logarithmic relationship between the hop storage index and the oxidation of hop α- acids and proposed a general formula to calculate the % of α-acids plus β-acids lost depending on the HSI. The formula is as shown below:

$$\% (\alpha + \beta) \text{ lost} = 101.8 \log (\text{HSI}/0.247) \quad \text{(Eq.14)}$$

Using Nickerson & Likens (1979) formula, overall losses in α- and β-acids for Chinook hops would be around 57% and 77% for air- or nitrogen-exposed samples respectively; whereas total acids lost in Cascade pellets would be around 40 and 43% for nitrogen and air-exposed samples respectively. These values are much lower than total a-acids lost at 35ºC for Chinook pellets which are 95.2% and 84.4% for nitrogen flushed and air-exposed pellets. While for Cascade total α-acids the losses at 35ºC are 87.7% and 63.9% nitrogen flushed and air-exposed pellets.

a) Cascade



b) Chinook

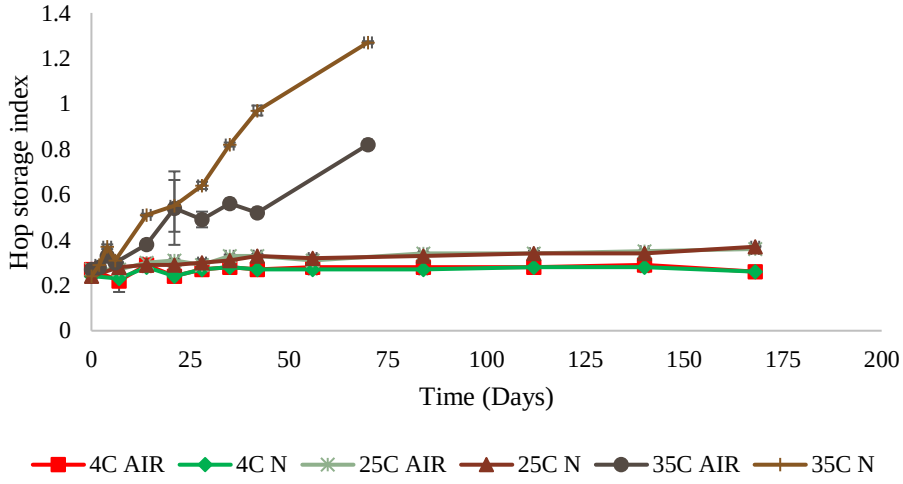


Figure 4.9 HSI values for (a) cascade and (b) chinook hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

4.4 Effects of Storage Temperature on Oil Content

The results from this thesis are reported in an as-is basis. As previously mentioned, a desirable moisture content after kilning hop cones is somewhere between 8 and 12% (Krofta, Mikyška, and Hašková, 2008). It is preferred that the moisture of hops be around 8% because as moisture content increases microbial activity will also increase. Furthermore, one study mentions that higher moisture content during storage could have a negative effect on stability in hops especially with respect to degradation of α- and β-acids (Tedone et al, 2020). In our study, the moisture content of the hop pellets averaged 12.3 and 12.1% for Cascade and Chinook pellets respectively, as shown in Table 4.1. The moisture content of the hop pellets increased from the start of the experiment to the end at 35ºC, where after 70 days of storage condensation of water was noticeable in the inside of the package and the moisture content increased to around 13% for both varieties. The increase in moisture content was observed in both variety samples as shown in Table 4.1. The increase in moisture content at 35ºC is correlated with the greatest degradation of α-acids as well as with a noticeable loss of essential oil. Since the samples were packaged in multi-laminated foil bags, the moisture transferred through the package may be considered negligible over 70 days, and the appearance of condensation in the inside of the package was likely due to the formation of water as a by-product of the degradation reactions in the pellets.

The total oil content of the hop pellets decreased with increasing storage time, and the decrease was greatest at 35ºC compared to 25ºC. There was a more significant loss in Chinook than in Cascade samples especially at high temperatures as shown in Table 4.1. The amount of oil lost correlates with the amount of α-acids lost. After 7 months of storage oil content loss at 35°C was 41.9% for Chinook and 25.6% for Cascade. However, even at the beginning of the study, both varieties had oil content well below the typical range of those varieties. Typical total oil content for Cascade is around 0.8 – 1.5 mL/100g, whereas for Chinook the oil content is typically higher ranging between 1.5 – 2.7 mL/100g (Healey, 2016). The low values observed in the hop pellets used for the present study may be due to conditions before harvest, postharvest processing and varietal factors. These factors affect the α- acid content in hops as well (Sharp, Townsend, Qian and Shellhammer, 2014). The content of hop oil increases through ripening. An early study by Howard and Slater (1958) showed that for a given season, the oil content of fresh Fuggle hops picked over 6 weeks, in early picked hops oil content increased from 0.15% to 0.3% in a period of

less than 4 days, whereas for hops picked after 5 weeks the content increased rapidly from 1.0 to 1.5% in less than 2 weeks. They also reported that during drying some samples showed a decrease in oil content from 1.24% to 0.8%, and that commercial drying of hops using a kiln dryer lowered the oil content from 0.80% to 0.54% (Howard and Slater, 1958). Therefore, ripeness at harvest in combination with adequate kilning has a considerable impact in the total amount of oil in the hops. In the present study, the oil content was determined for pelletized hops, so the amount of oil in fresh hops is unknown. It would be useful in the future to determine the oil content for fresh hops as well as for dried hops, in this way, it would be possible to know if the low contents observed were due to kilning, especially since this was the first time that this farm used their newly installed kiln dryer.

A relationship between the losses in α-acids and myrcene content has been documented. It is believed that varieties with initial high amounts of α-acids will suffer a great loss of α- acids and their oxidation is related to the essential oil content especially myrcene in the hop samples (Hartley, 1967; Menary, Williams and Doe, 1982). In a study, Hartley (1967), found that seeded Bullion hops had a high amount of initial essential oils and the highest loss of α- acids during storage, but Early Cluster hops which had the lowest amount of initial essential oils had the lowest percentage loss of α- acids. In contrast to this, Tedone et al., (2020) investigated two varieties Galaxy and Vic secret which both shared high amounts of α-acids and essential oils but behaved differently in their loss of percentage α-acids and β-acids that is, though they both had high amounts essential oils Galaxy had greater α- and β- acids loss compared to Vic secret.

Table 4.1 Table showing moisture content and oil content of Hop pellets after 7 months of storage

Variety	Storage Temperature (°C)	Moisture content (%)		Oil content (mL/100g)	
		Initial	Final (7 months)	Initial	Final (7 months)
Cascade	4	12.3 ± 0.7	12.8	0.39	0.39
	25	-	11.8	-	0.32
	35	-	13.2	-	0.29
Chinook	4	12.1 ± 0.7	11.6	0.74	0.74
	25	-	12.6	-	0.59
	35	-	13.4	-	0.43

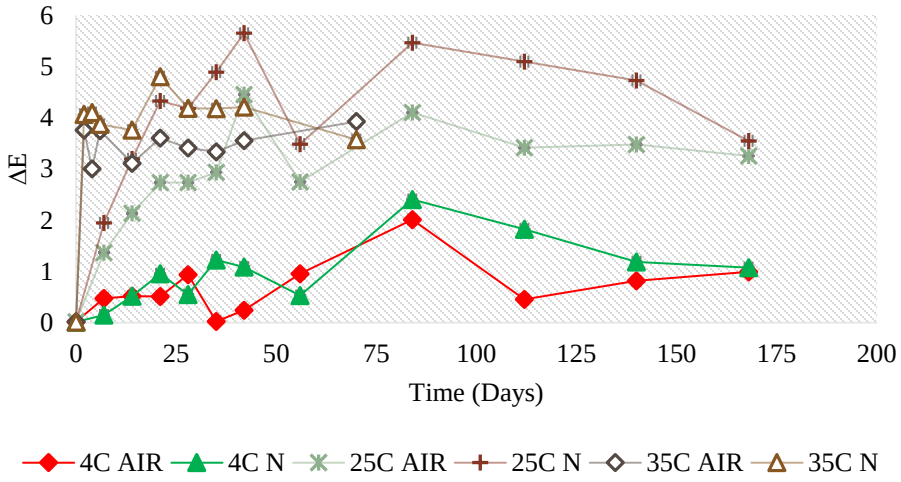
4.5 Effects of Temperature on Color

Results for color of hop pellets sample as a function of storage time and temperature are shown in Figures 4.10 to 4.15. During storage it was observed that at 4°C the total color difference (ΔE) was stable except for day 84 when there was a peak in the total color difference. This was observed for Cascade samples exposed to air and those exposed to nitrogen, it was also observed in Chinook hops pellets at 4°C that were flushed with nitrogen. At 25°C for both varieties there was a sharp increase from the initial ΔE value until day 21, another spike in the values was observed at day 42. For the Cascade variety at 25°C after day 42 there was a gradual decline in ΔE. However, for Chinook at 25°C the ΔE values remained stable until the end of the experiment, this is observed in Fig. 4.10 b. Color difference at 35°C for Cascade had a sharp increase from the initial value to day 2 for both nitrogen-flushed and air exposed samples. From day 2 the nitrogen flushed samples values became stable and experienced another sharp increase at day 35, after day 35 ΔE values remained stable again. Air exposed Cascade samples had fluctuating values from day 2 to day 6, after which ΔE values remained stable until there was a slight decrease on day 70. Also at 35°C, for Chinook samples there was a sharp increase in ΔE for day 2 for both air exposed and nitrogen flushed samples, ΔE values then remained stable until there was a slight increase in the color

difference values for day 70, as seen Fig 4.10. The three color parameters, L*, a* and b* showed some variation during storage. The observed trends were the same in both varieties. L* value refers to the lightness of the sample and indicates how white or black it is. L* is measured in a scale that ranges from 0 to 100, where 0 corresponds to black and 100 corresponds to white. The a* values can either be negative (-) or positive (+), the (-) a* value refers to the greenness of the sample while (+) a* value refers to redness. The negative (-) b* value refers to how blue a sample is while the yellow color is shown by a positive (+) b* value (Fellows, 2017; Nielsen, 2010). Throughout the storage period, the differences observed for the values of color parameters followed a similar trend for both varieties under both atmospheric conditions. The effect of gas type was not significant within varieties, except for Chinook samples stored at 35^{o}C at 42 and 70 days where samples stored under nitrogen and under air showed significant differences. For the L* values the lightness or the darkness had slight changes at 4°C and remained stable for the whole duration of the experiment, this was observed in both varieties. At 25°C and 35°C the values were stable within the temperatures for 168 days and 70 days respectively. However, compared to 4°C the values were slightly higher. Though the L* values were stable within the same temperatures the values were slightly higher at higher temperature (25°C and 35°C). This was observed in both varieties as shown in Fig 4.11. The b* value was also stable at 4°C for Cascade and Chinook for 168 days. For 25°C the b* value remained stable for 168 days, but those values were slightly higher than those of 4°C, as shown in Fig 4.12. At 35°C in both varieties the b* value was stable but just like at 25°C the values were slightly higher than the values at 4°C. In all varieties the a* values at 4°C were stable. As the temperature increased at 25°C there was a sharp increase on day 7 and from there the values remained stable. At 35°C there was also a sharp increase in the a* values but higher than the increase at 25°C this increase was observed at day 2. After that the values were stable until day 42 where there was another increase to day 70, as shown in Fig. 4.13. Individual color parameters provide an objective indication of the color stability of the sample, but do not provide information about whether those differences would be perceived by a person. Thus, color parameters L*, a*, b* were used to calculate the chroma or saturation, the hue angle, and the total color difference in the samples. Graphs of hue angle and Chroma of samples as a function of storage time for various temperatures are shown in Figs. 4.14 - 4.15. The hue is the perceived color of a sample (Fellows, 2017). In both varieties, atmospheric conditions and all temperatures, the

hue angle decreased with increase in temperature and time (Fig. 4.14). From these results the perceived color of the hops changed at higher temperatures. As the perceived color of hops is usually green the reduction in the hue angle indicates that the hop samples were perceived to be less green with time. For Cascade samples at 4°C the hue angle for both nitrogen-flushed, and air exposed samples fluctuated around 90° and 91°, while for Chinook the fluctuation was around 89° and 90°. There was a sharp decrease in hue angle at day 2 and day 7 for 35°C and 25°C respectively. The decreases were not equal, as the decrease at 35°C was greater than in 25°C, after day 7 at 25°C the hue angle became stable until day 168, while at 35°C there was a steady decrease of the hue angle until day 42 followed by a sharp decrease observed at day 70. Hue angle values for Cascade samples stored under nitrogen or air were 84.2^{o} and 83.7^{o} respectively at 35^{o}C; whereas for Chinook samples the hue angle values were 81.9^{o} and 83.9^{o} for samples stored under nitrogen or air respectively. The chroma is the saturation of the color and increases with more pigmentation and reduces as the sample darkens (Nielsen, 2010). The chroma of the samples was stable at all temperatures with little reduction or increase with time. Though there was stability, at different temperatures there was a variation in the chroma. At higher temperatures (25°C and 35°C) there was a slight increase in chroma compared to the chroma values at 4°C. This was observed in both the hop varieties (Fig 4.15a – 4.15b). Though the trends were similar for all the color components (L*, a* and b*) there was a difference in how both varieties responded to the increase in temperature during storage. For these components, Chinook showed greater degradation at 35°C, the differences in the L* and b* values were not significant, however, for the a* values, Chinook and Cascade had insignificant differences at 35°C except for nitrogen-flushed Chinook samples. For both varieties the overall change in color was insignificant, indicating that the change could not be easily detected by the naked human eye. As the study progressed the samples especially those at higher temperature showed that they were less green, compared to the samples stored at 4^{o}C which seemed to keep the same color after 168 day. Wain, Baker and Laws (1978), reported that after 43 weeks of storage of Northern Brewer and Styrian Golding hop pellets stored in sound packs it was observed that the hops had turned light brown and did not have off flavors. As for the pellets stored in ruptured bags, they were browner with a cheesy aroma.

a) Cascade



b) Chinook

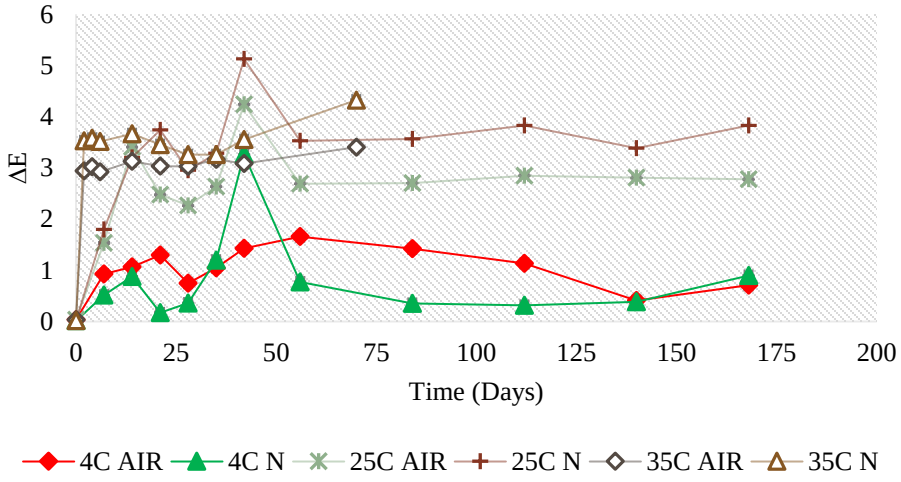


Figure 4.10 Total color difference (ΔE) for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

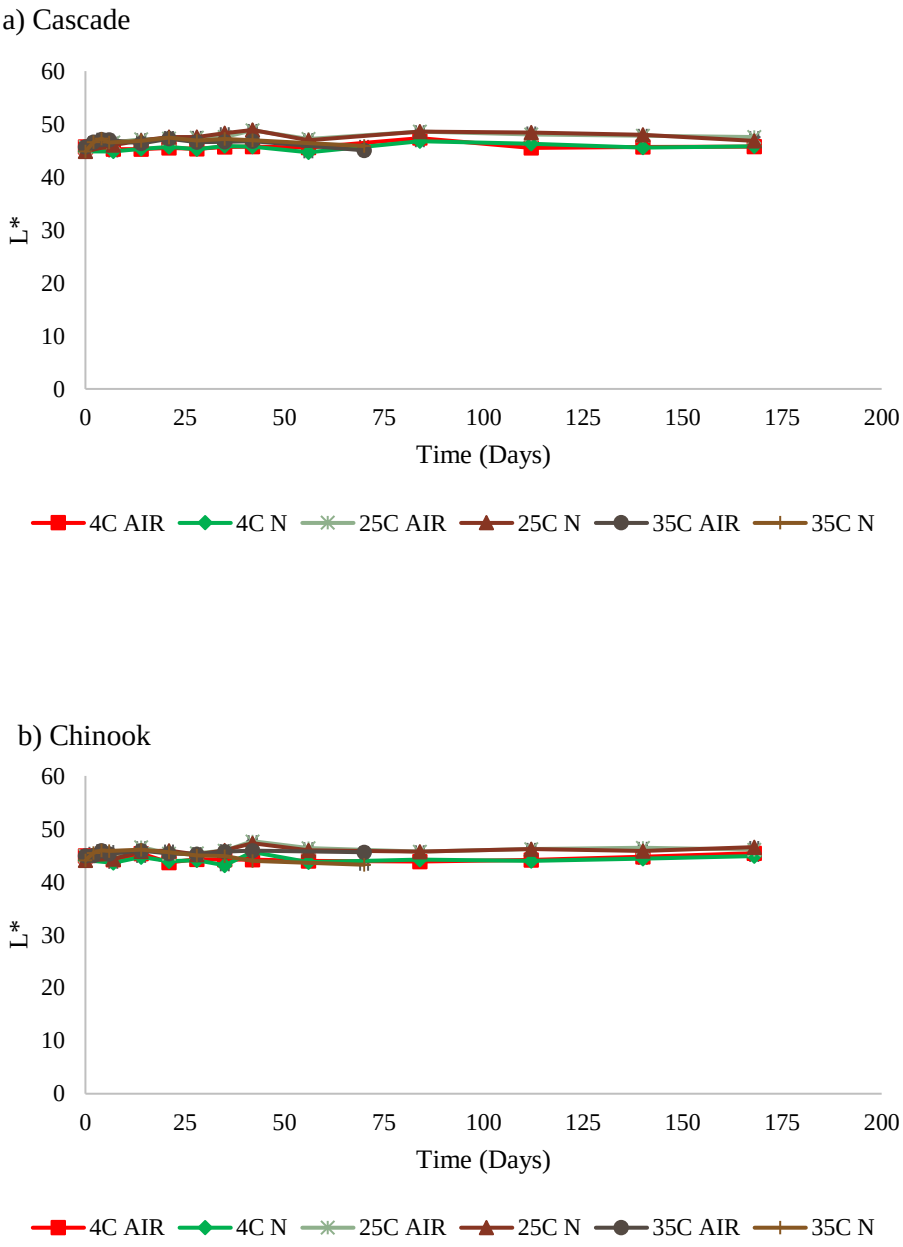


Figure 4.11 L* values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

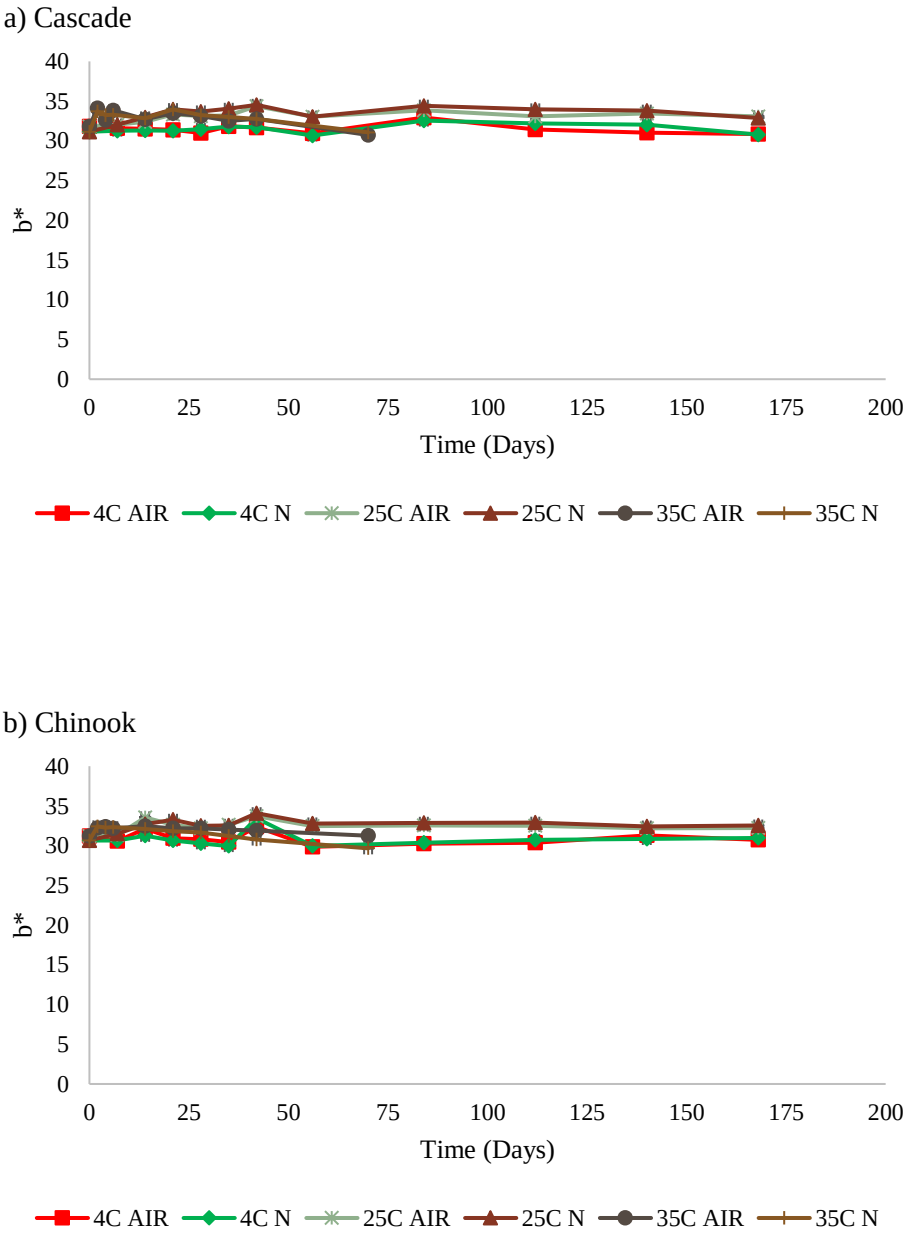
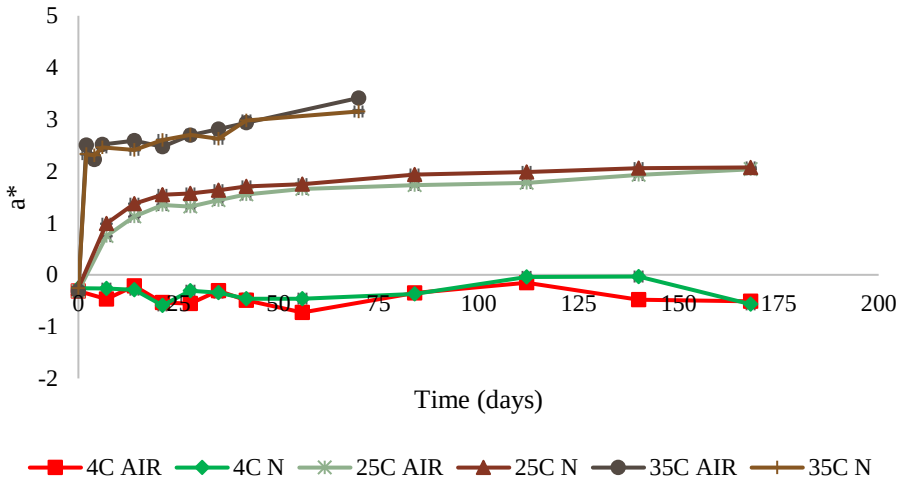


Figure 4.12 b* values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

a) Cascade



b) Chinook

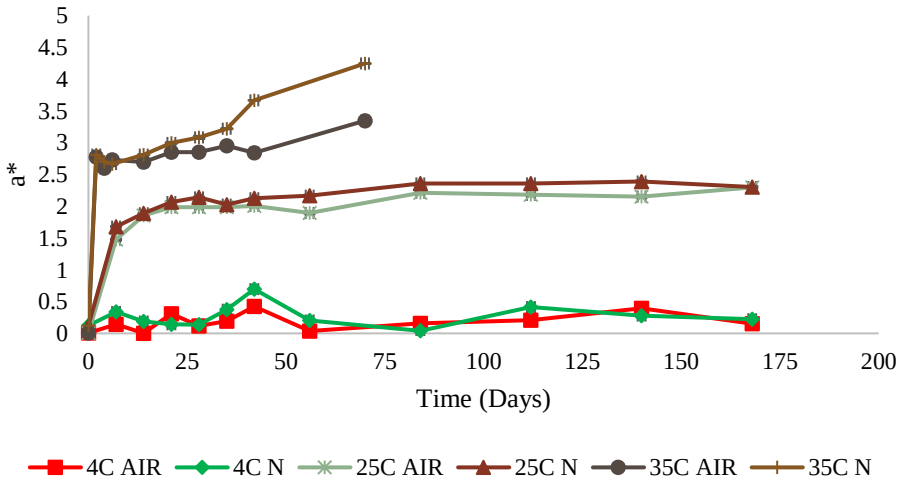


Figure 4.13 a* values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

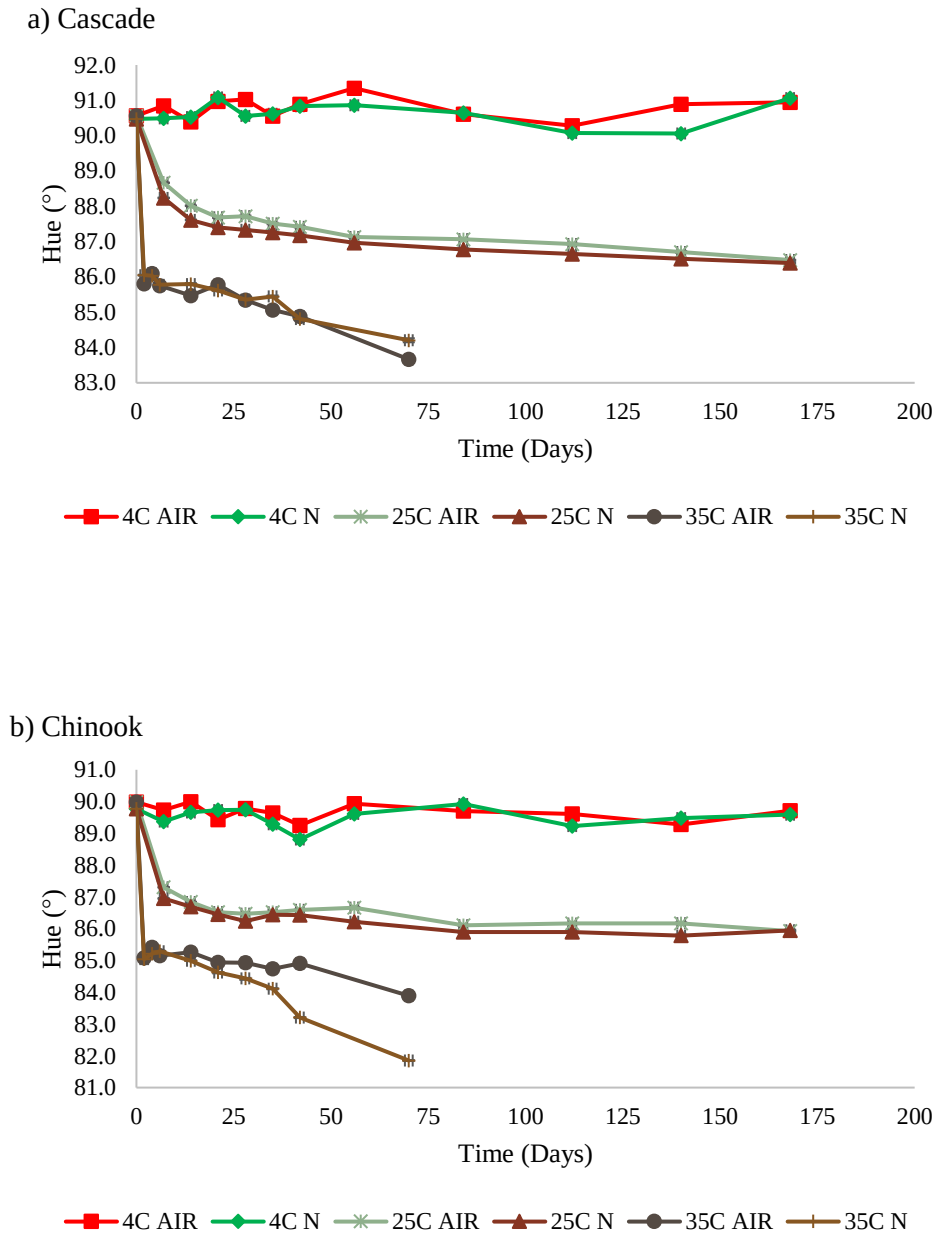


Figure 4.14 Hue angle (°) values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

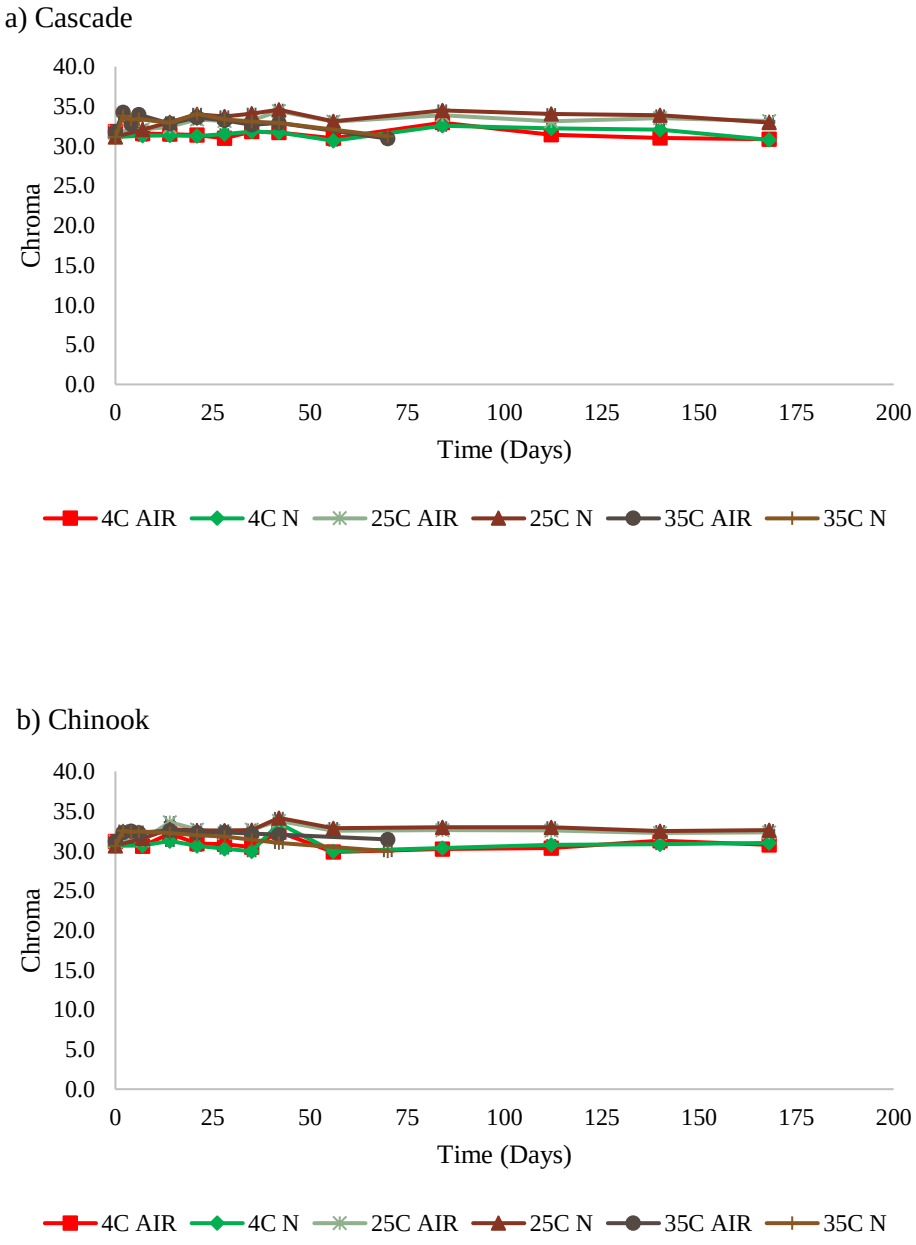


Figure 4.15 Chroma values for Cascade (a) and Chinook (b) hop pellets stored in nitrogen flushed and air exposed atmospheric conditions. Samples were stored at 4°C, 25°C for 168 days and 35°C for 70 days of storage. Values represent average of triplicate analysis ± standard deviation.

4.6 Degradation Rate Constants of α-Acids and β-Acids

Experimental results were fitted by linear regression to determine the order of the reaction that best described the decrease of α- and β-acids at each temperature as a function of storage time. For this purpose, graphs of concentration (C) or natural logarithm of concentration (ln C) or inverse of concentration (1/C) against time were constructed. The coefficient of determination (R^2) was used to determine the best fit.

In his study, Green (1978) determined that the loss of α–acids followed a first order kinetic equation meaning that the α–acids amount lost are proportional to the amount present in the hop pellets and that their loss follows an exponential law. Results from our study show that α–acids from both varieties, Cascade and Chinook, stored at 35°C followed a first order degradation kinetics as shown in Fig. 4.16 and 4.17. From the storage trials of Wye hops Green (1978) explained that the correlation coefficients showed which equation gave the best fit, and although the difference between zero order and first order was not significant, the first order equation was preferred. Similarly, Skinner et al. (1977) reported that baled Pride of Ringwood hops stored at -20, -5, +5 and +22^{o}C were adequately modeled following either zero or first order kinetics.

For a given set of data points, the difference between modeling data using a zero order or a first order equation become more noticeable at longer storage times, since the initial portion of an exponential decay curve is practically linear (Skinner et al., 1977). It is possible that depending on the number of experimental points, if those were collected at early stages, predicted values from both equations could overlap. However, it is at later stages that assumption of a zero order reaction would over-estimate the losses if the data in reality followed first order kinetics; whereas if the data truly followed a zero order kinetic equation but predictions were obtained using a first order equation, the predicted losses would be underestimated. More recently, Malowicki and Shellhammer (2005) also reported that α–acid loss and iso–α-acids loss follow first order reaction kinetics. While at 35^{o}C, the loss in α–acids followed the first order reaction, data fitting at 25°C showed lower values for R^2, but still, higher R^2 values were obtained for first order equation than for zero or second order equations. On the other hand, at 4^{o}C, the concentration of α-acids did not change significantly over time, therefore, the value of the slope was nearly zero, and the model was not significant. The individual components of α–acids (cohumulone, N + adhumulone)

exhibited the same trend as total α–acids as shown in Fig. 4.18 to 4.21. The same trends were observed for Chinook pellets. The beta acids remained mostly stable during the duration of the study as previously shown in Fig. 4.5 – 4.8. So, most of the relations were not significant, since the slopes were very close to zero, indicating no degradation, however slight trends were observed. After the reaction order for degradation of total and individual components was determined, the kinetic rate constant at each temperature and for each component was obtained from the slope of the regression lines of the natural logarithm of the concentrations *versus* time. First order degradation rate constants are shown in Table 4.2. Data fitting showed that for β-acids at 4°C and 25°C there was no effect of any of the factors: gas type, temperature, storage time. However, for Cascade samples stored under air and for Chinook samples stored under nitrogen, the degradation rate constant at 35°C was significantly different than degradation rate at 4°C, whereas degradation rate at 25°C was not significantly different from either 4 or 35°C. Closer examination of degradation rate constants indicates that a slight degradation of colupulone was observed even at low temperatures. Green (1978), explained it as hops being in their delay period as a reason why they had not shown enough degradation in the first 6 months of storage, it was further on explained that hops have different delay periods for their hops. The delay period meaning that degradation was stationary or too little to carry out a kinetic analysis. The degradation rate values obtained in our study were approximately one order of magnitude (10x) lower than those reported by Skinner et al. (1977). They reported degradation constant for α–acids of -7.2×10^{-3} and -1.89×10^{-2} for baled hops stored at 5°C and 22°C respectively.

Knowledge of the degradation rate constants of hop components is important to model the decay over storage time; however, knowledge of the dependence of the rate constant on temperature is equally important to predict how components may degrade due to fluctuation in storage temperature. The Arrhenius equation is an equation that has been used in the past to model the dependence on the temperature of the kinetic rate constants (k). In general, higher temperature leads to an increase in the value of k. While the Arrhenius equation is an empirical model, i.e. it does not provide a mechanistic explanation of the reaction under study, it has been successfully used to model various phenomena. Once the pre-exponential factor or the value of k at a reference temperature and the activation energy of certain process is known, the equation is useful to predict degradation rate constants at different temperatures and thus, the concentration of a component at

any given time. Knowledge of degradation rate constant as a function of temperature is useful to determine, for example, the storage life or the shelf life of various ingredients or even foods. The validity of Arrhenius-type relationships over broad range of temperatures will depend on the phenomenon under study. The kinetic constants determined for total α-acids, total β-acids, humulones and lupulones were fitted using an Arrhenius type relationship. Interestingly, only lupulone degradation showed a high coefficient of determination. Indicating that its degradation may be predicted through the entire range of temperatures. The values of activation energy obtained for the total and individual components of α– and β–acids are shown in Table 4.3. Results indicate that the Arrhenius equation was useful to predict the temperature dependence of k only for certain samples, but in the cases that it was adequate, the value of the coefficient of determination was greater than 0.9. The results also show that the value of the activation energy is larger for Chinook pellets than for Cascade pellets. Skinner et al. (1977) reported activation energy values of 27.42 kJ/mol (6.55 kcal/mol) for Pride of Ringwood baled hops, which was much lower than the values obtained in our study 90-95 and 114-115 kJ/mol for Cascade and Chinook hops respectively. Skinner et al. (1977) calculated that the rate of deterioration would double for every 15^{o}C rise in storage temperature, whereas in our study, the rate of deterioration would increase about 9.8 times or 12.8 times for Cascade and Chinook hops with a 10^{o}C change in temperature (k_{35C}/ k_{25C}). These results indicate that α–acids in Chinook hops are more sensitive to changes in storage temperature than Cascade hops.

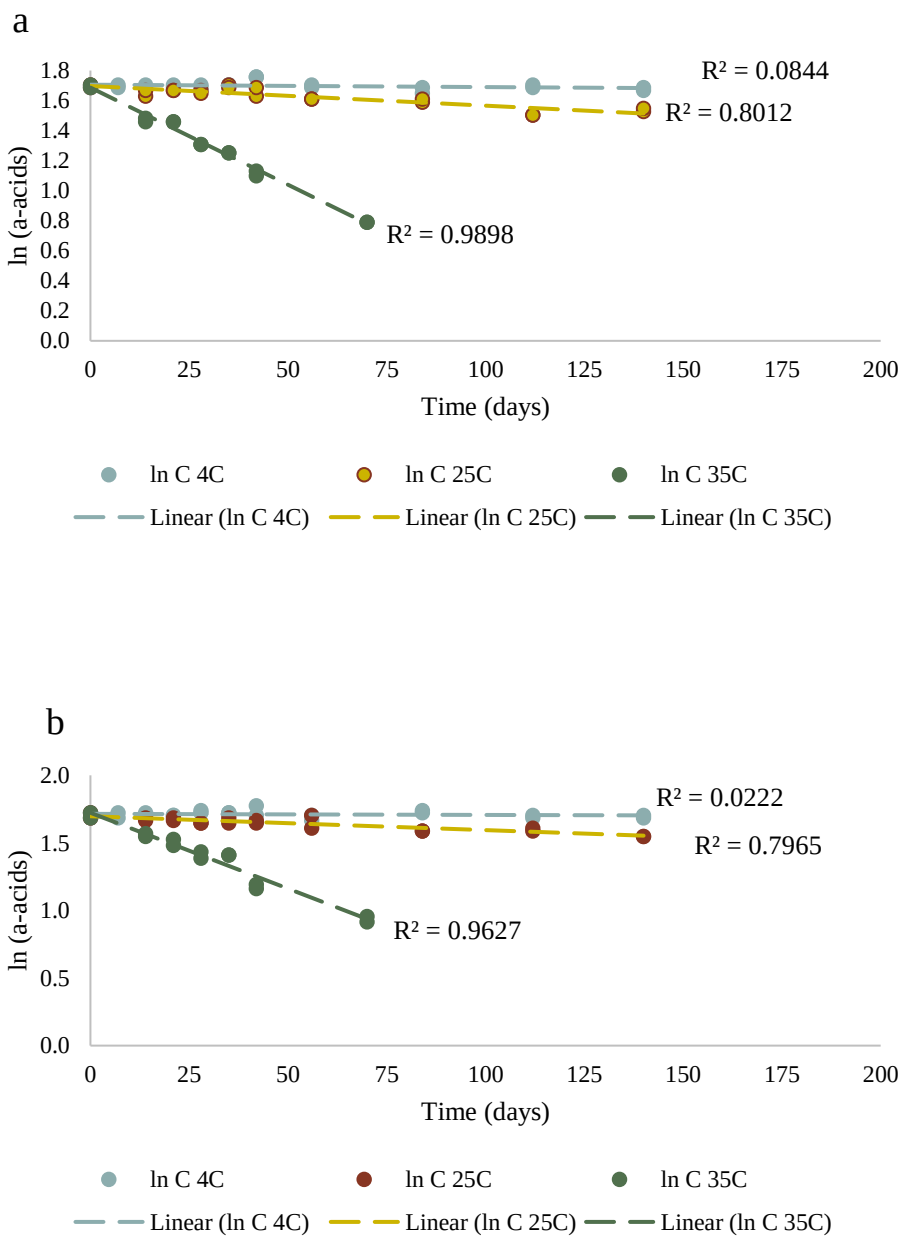


Figure 4.16 First order reaction correlation for degradation of total α-acids for Cascade hop pellets stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation.

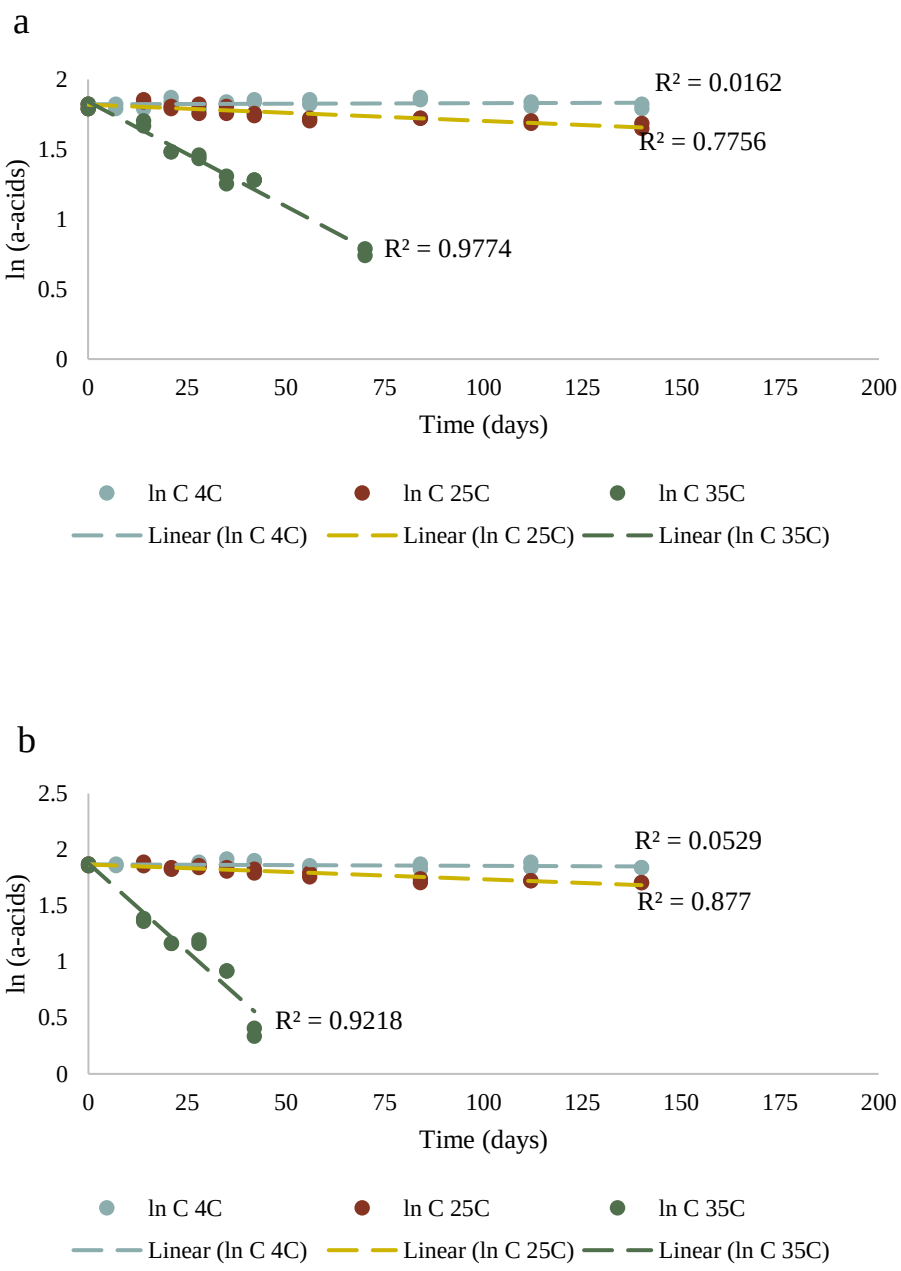


Figure 4.17 First order reaction correlation for degradation of total α-acids for Chinook hop pellets stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation.

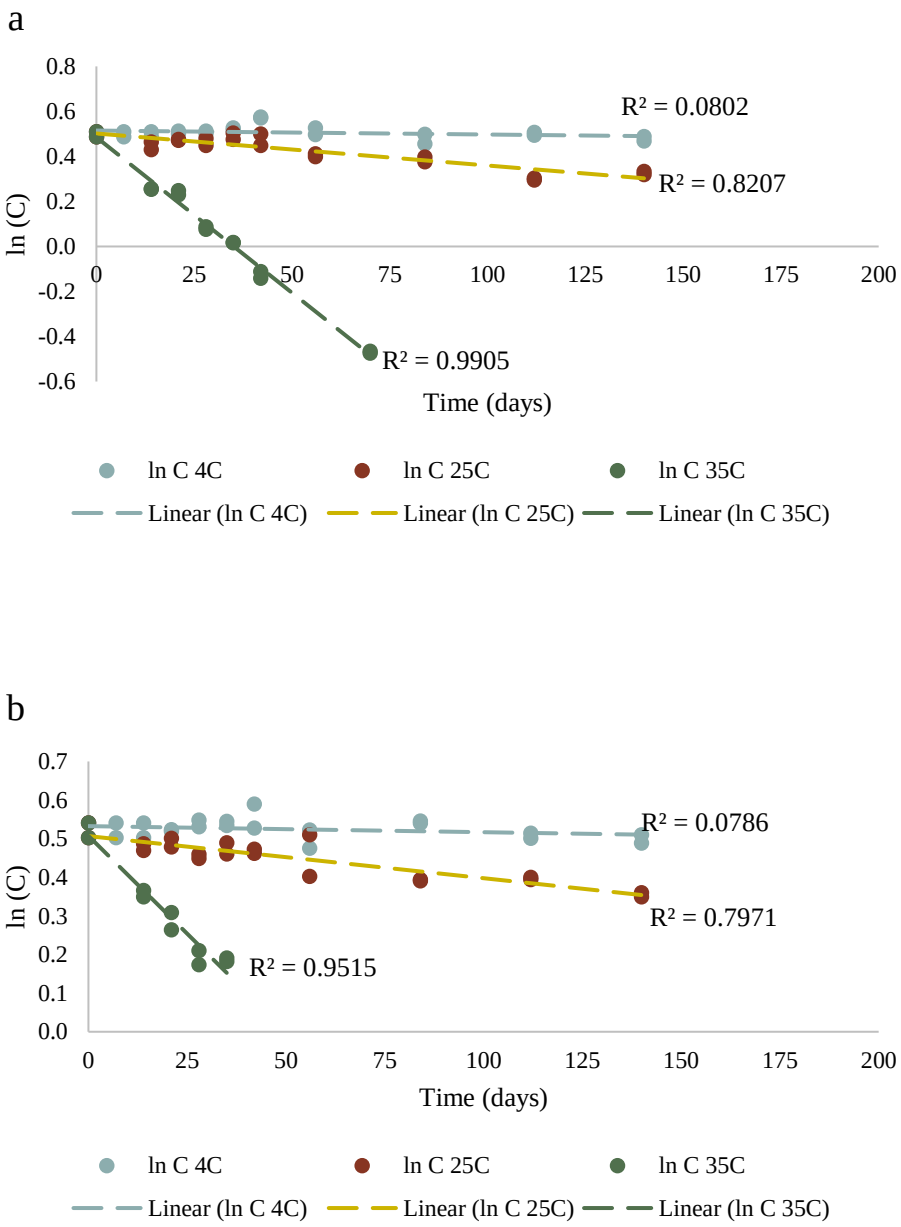


Figure 4.18 First order reaction correlation for degradation of cohumulone component for Cascade stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation.

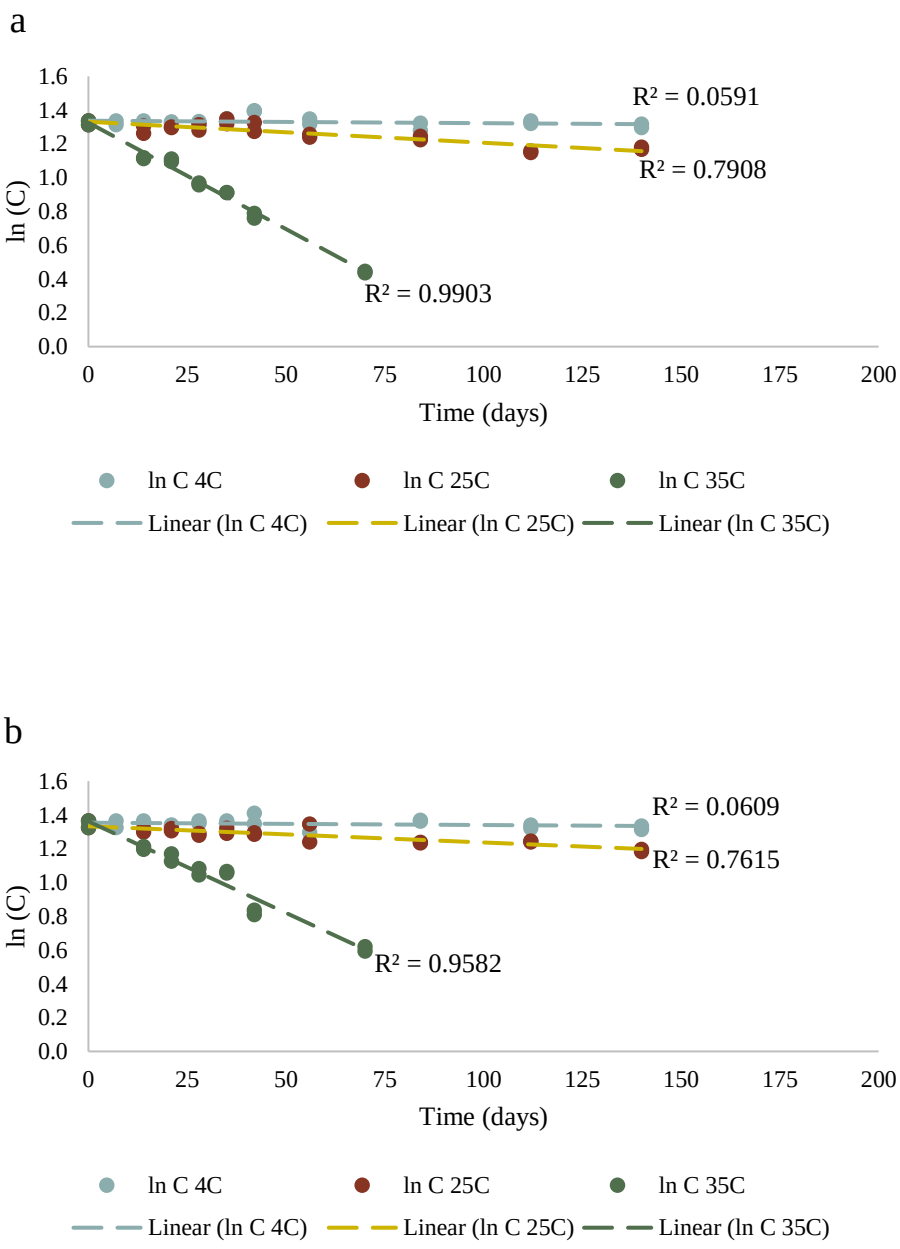


Figure 4.19 First order reaction correlation for degradation of N + adhumulone component for Cascade stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation.

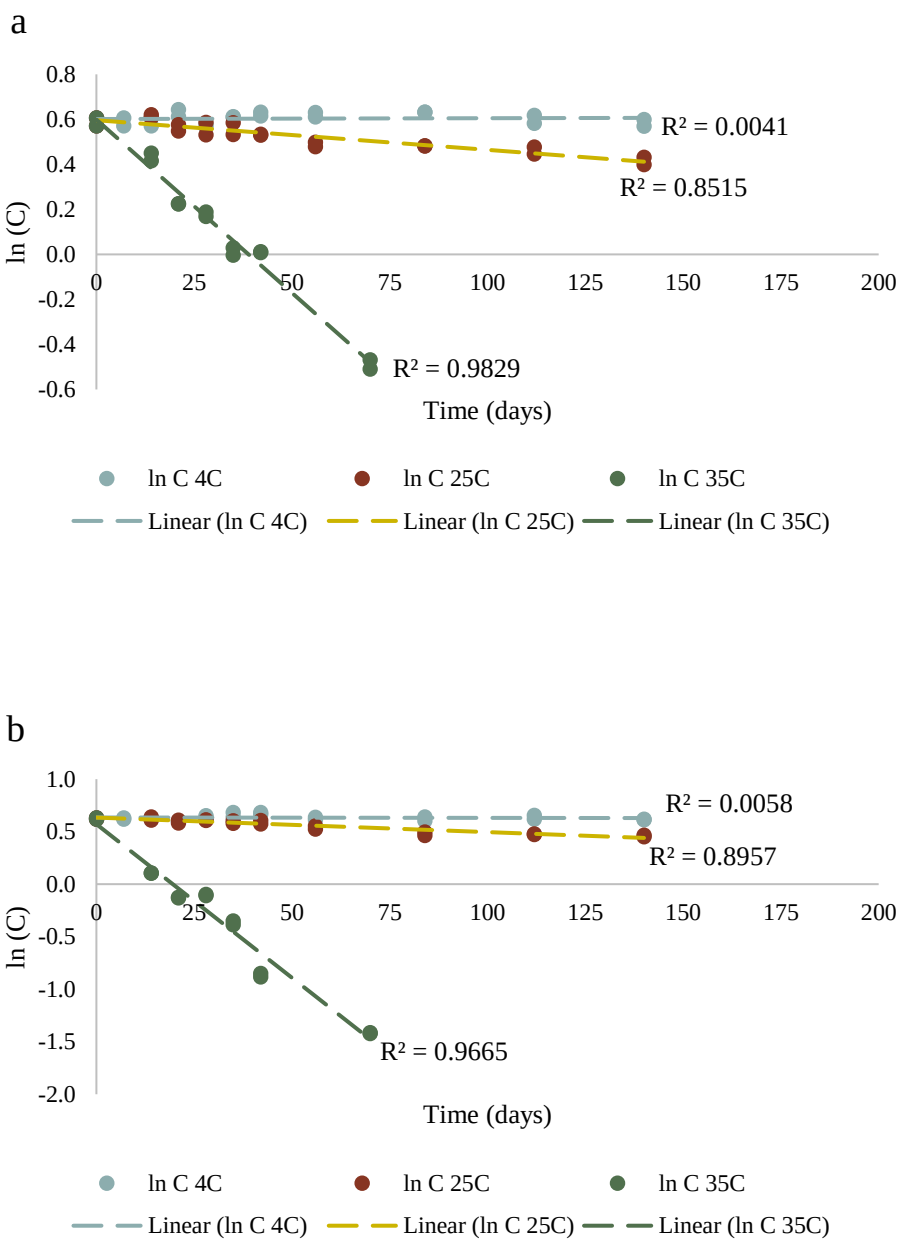


Figure 4.20 First order reaction correlation for degradation of cohumulone component for Chinook stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation.

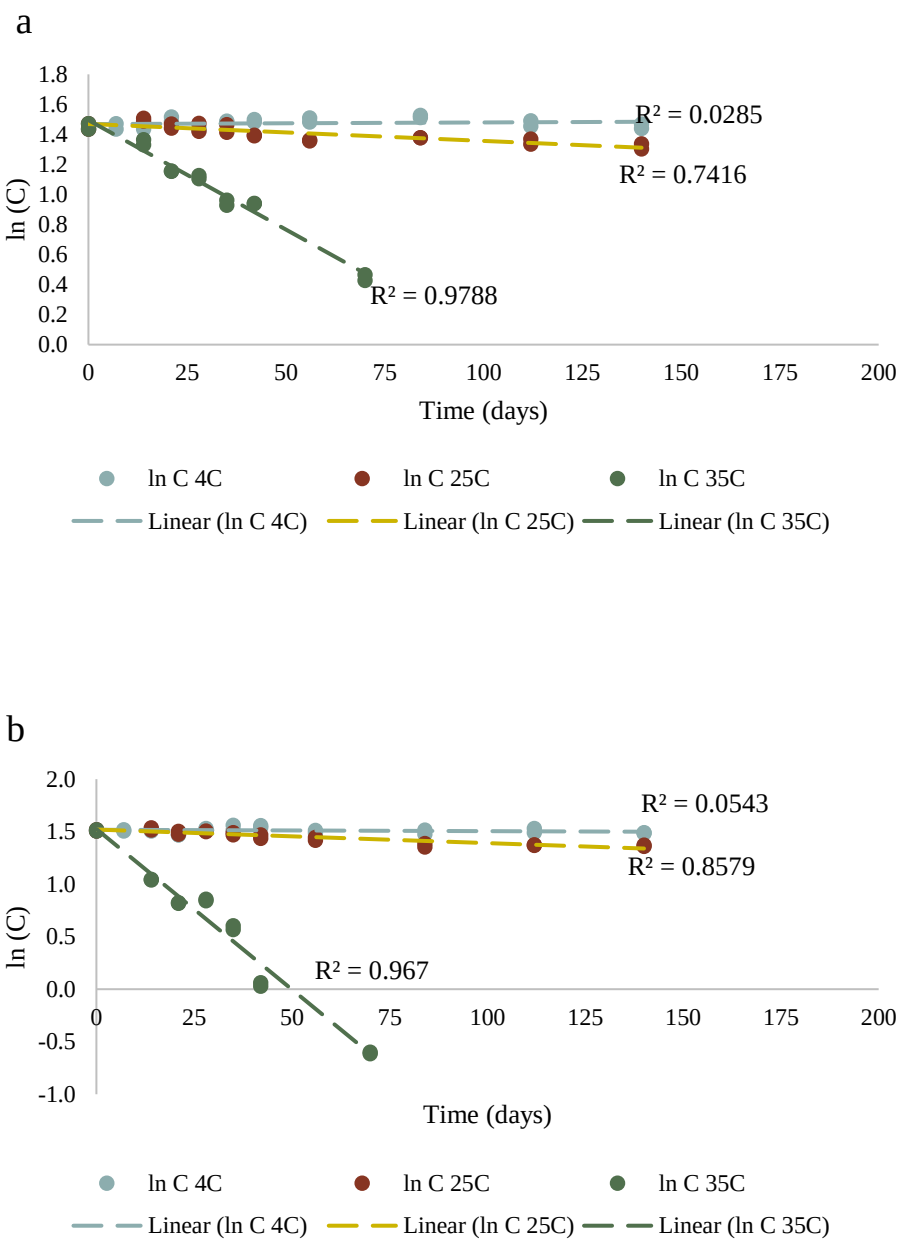


Figure 4.21 First order reaction correlation for degradation of N + adhumulone for Chinook stored under (a) air or (b) nitrogen at 4°C, 25°C or 35°C as a function of time. Values represent average of duplicate analysis ± standard deviation.

Table 4.2 Average first order degradation kinetic constant (k) for total a-acids, cohumulone, n + adhumulone, total b-acids, colupulone, and n + adlupulone measured by HPLC for hop pellets stored at 4, 25, and 35°C under air or nitrogen gas in the headspace. MSE is the mean square error of the data. Values represent average of duplicate analysis ± standard deviation.

Component	Temperature (°C)	Cascade			Chinook		
		Air	N_2	MSE*	Air	N_2	MSE*
		k (day^{-1})	k (day^{-1})		k (day^{-1})	k (day^{-1})	
Total α-acids	4	-1.58E-04	-1.63E-04	± 7.1 E-6	7.80E-05	-1.32E-04	± 2.01E-04
	25	-1.31E-03	-1.03E-03		-1.17E-03	-1.32E-03	
	35	-1.29E-02	-1.11E-02		-1.50E-02	-3.01E-02	
Cohumulone	4	-1.76E-04	-2.52E-04	± 7.4 E-6	3.60E-05	-4.20E-05	± 2.01E-04
	25	-1.42E-03	-1.09E-03		-1.32E-03	-1.38E-03	
	35	-1.38E-02	-1.21E-02		-1.54E-02	-2.92E-02	
N + Adhumulone	4	-1.45E-04	-2.15E-04	± 7.2 E-6	1.11E-04	-1.33E-04	± 5.58E-04
	25	-1.24E-03	-9.59E-04		-1.13E-03	1.70E-05	
	35	-1.26E-02	-1.08E-02		-1.46E-02	-3.06E-02	
Total β-acids	4	+0.00E+00	-6.00E-05	± 5.9 E-6	-3.13E-04	1.18E-04	± 2.10E-04
	25	-1.80E-04	-1.70E-05		-3.08E-04	-4.38E-04	
	35	-3.70E-04	-1.57E-04		-4.54E-04	-1.41E-03	
Colupulone	4	-4.90E-05	-1.20E-04	± 7.8 E-6	-2.75E-04	2.40E-05	± 2.03E-04
	25	-2.53E-04	-4.30E-05		-3.18E-04	-4.60E-04	
	35	-5.41E-04	-2.97E-04		-6.90E-04	-1.68E-03	
N + Adlupulone	4	+4.60E-05	-2.30E-05	± 6.8 E-6	-3.46E-04	7.30E-05	± 2.03E-04
	25	-1.93E-04	-2.10E-05		-2.55E-04	-4.48E-04	
	35	-3.53E-04	-1.06E-04		-4.96E-04	-1.50E-03	

Table 4.3 Activation energy and coefficient of determination for total α-acids, cohumulone, N + adhumulone, total β-acids, colupulone, N + adlupulone calculated from fitting experimental results with the Arrhenius equation.

Component		**Cascade**		**Chinook**		**Units**
		Air	**N_2**	**Air**	**N_2**	
Total α-acids	E_a R^2	95.3 0.94	90.2 0.91	114.7 0.96	115.7 0.90	kJ/mol
Cohumulone	E_a R^2	94.3 0.94	81.4 0.87	135.1 0.99	146.3 0.97	kJ/mol
N + Adhumulone	E_a R^2	96.6 0.94	82.5 0.84	105.3 0.94	90.5 0.27	kJ/mol
Total β-acids	E_a R^2	55.0 0.97	10.8 0.05	6.9 0.50	54.3 0.96	kJ/mol
Colupulone	E_a R^2	54.8 1.00	11.1 0.07	18.2 0.68	97.2 1.00	kJ/mol
N + Adlupulone	E_a R^2	46.7 1.00	6.8 0.49	5.0 0.11	67.4 0.99	kJ/mol

CHAPTER 5. CONCLUSION AND RECOMMENDATIONS

Control of hop storage conditions is important in maintaining the quality of hop pellets. Hops need to be stored in conditions that will preserve their most valuable constituents primarily the bittering compounds or α-acids. When stored under refrigeration temperature of 4°C the α-acids in hop pellets degrade much more slowly than in hops stored at high temperatures (25 or 35°C). Although the extent of deterioration in hops is variety dependent, cold temperatures help to slowdown degradation regardless. The packaging of hops is also important in maintaining the quality of hops. Multi-laminated bags that are sound, not letting air or light in are an added advantage to keeping pellets fresh. Control of degradation reactions ensures that α-acids are retained in hops throughout storage, meaning that the hops maintain their brewing value and bittering potential during the boiling step in brewing.

The objective of this project was to evaluate the changes in concentration of hop α-acids, β-acids, hop storage index, color and oil content of hop pellets stored under various temperatures (4°C, 25°C, 35°C), package atmospheric composition (N_2, air) in Cascade and Chinook hop varieties grown in central Indiana in 2019. To achieve this objective the impact of hop aging on the individual hop chemical constituents was determined for approximately 6 months.

When hops were stored at 4°C there was no significant degradation observed in α-acids for up to 168 days. Also, at 4°C, Cascade and Chinook hop pellets stored under air or nitrogen in the package headspace had no significant impact in the loss in α-acids. At 25°C the degradation rate of α-acids was slightly higher than at 4°C for both Cascade and Chinook samples. Hops can be stored at this temperature for a short period of time without getting spoiled. At 35°C there was a significant loss of α-acids compared to 4°C and 25°C in Cascade and Chinook samples. Hops should not be stored or transported at this temperature as it will cause rapid degradation of α-acids.

The effect of hop variety and atmospheric composition in headspace was not significant at either 4 or 25°C. However, at 35°C the impact of atmospheric composition was significant in the degradation of Chinook hop pellets, where nitrogen-flushing seemed to favor the degradation of

α-acids. The observed effect was most likely the result of an anomaly, since it was only observed for the last two samples of Chinook pellets analyzed, and gas headspace composition was not significant in the degradation of Cascade hop pellets.

The HSI of the pellets showed a correlation with α-acid loss, as α-acids content decreased, the HSI increased continuously. Chinook hop pellets reached higher final HSI values than Cascade at 35°C. Hop pellets stored at 4°C and 25°C had values less than 0.4 and remained of brewing value even after being stored for 168 days. Therefore, it would be possible to store hops at room temperature for less than 168 days and still have pellets with good brewing value.

The total color difference was minimal at different temperatures but when hop pellets stored at 4°C were compared with those stored at 35°C a slight difference was observed. Storing hops at 35°C for 70 days did not accelerate color degradation in the hops. The initial color and final color of the hop pellets were not significant. The Chroma was stable for the duration of the study, but the hue angle experienced a decrease as the temperature increased to 25°C and 35°C. The difference of the hue angle became insignificant over time within these temperatures. The observations in the color were common to the two hop varieties.

The oil content of the hops reduced with increasing time and temperature and the reduction was most severe at 35°C. The reduction in essential oil content would be more detrimental to aroma hops, such as Cascade, since the essential oil contributes a characteristic hoppy aroma to beer. The decrease in essential oil would not be very important for bittering hops, since those hops are typically added early on during kettle boil and would be mostly evaporated anyway.

The second objective of this research was to determine the kinetic order for the decrease in concentration of α-acids in hops over storage time. The degradation of hop α-acids was found to follow the first order reaction kinetics. The kinetic order was clearly observed in the results from samples stored at 35°C. At 4°C the degradation extent was not enough to determine kinetic

modeling. At 25°C, the degradation rate was not significantly different from neither 4 nor 35°C. Examination of the rate constants (k) at the three temperatures showed a difference of 100x between degradation at 4 or 35°C. Determination of the rate constant as a function of temperature would be important to know the shelf life of these hops at temperatures either than the ones in the study. First order kinetic model implies that the concentration of the component of interest at any given time is a function of its concentration, therefore, the higher the concentration, the greater the observed losses at any given time. In this work, Chinook pellets had a slightly higher initial content of α-acids content than pellets from Cascade variety.

Overall, this study showed that refrigerated storage under inert atmospheres will result in hop pellets that retain a very high brewing value. However, storage at room temperature, might be acceptable particularly if the hops will be used within a few weeks. It was also shown that although hop color is not a critical characteristic to hop quality, its rapid degradation could be used as an indication that hops have been exposed to temperature abuse.

Future research recommendations include:

- More hop varieties should be collected from more than one hop farms to compare the hop chemistry and how similar varieties from different farms behave during degradation. Also, comparing hops grown in Indiana and those grown in other regions of the United States of America
- Investigate the harvest and post-harvest practices carried out by hop growers and determine if these practices can affect the hop chemistry and how these practices affect the hop chemistry during storage.
- Carry out a microbial study to investigate the reason behind the swelling of hop bags during storage and determine essential hop oils from the beginning of the study and throughout the study to have a clear picture of what really happens to oils during storage.
- Adjusting the nitrogen flushing time and use of larger storage bags to increase headspace and hence observing if the measured chemical contents will have a significant difference between nitrogen flushed and air exposed samples.

- Increase of storage duration to be able to observe a degradation in other hop constituents such as β-acids.
- It would also be helpful to brew beers using fresh hops and aged hops and carry out chemical and sensory analysis on the beers to better understand the impact of hop age on the beers made with these hops.
- To observe a degradation in hops at low temperatures they should be stored for more days than they were in this study

REFERENCES

Alderton, G., Bailey, G. F., Lewis, J. C., & Stitt, F. (1954). Spectrophotometric determination of humulone complex and lupulone in hops. *Analytical Chemistry*, *26*(6), 983-992.

Almaguer, C., Schönberger, C., Gastl, M., Arendt, E. K., & Becker, T. (2014). *Humulus lupulus*–a story that begs to be told. A review. Journal of the Institute of Brewing, 120(4), 289-314.

Alonso-Esteban, J. I., Pinela, J., Barros, L., Ćirić, A., Soković, M., Calhelha, R. C., ... & Ferreira, I. C. (2019). Phenolic composition and antioxidant, antimicrobial and cytotoxic properties of hop (*Humulus lupulus* L.) Seeds. Industrial Crops and Products, 134, 154-159.

American Homebrewers association, 2014, How to store Homebrew Ingredients Retrieved from https://www.homebrewersassociation.org/how-to-brew/store-homebrew-ingredients/#:~:text=Whole%20hops%20under%20the%20same,(the%20colder%20the%20better) Retrieved on retrieved 10/28/2020

American Society of Brewing Chemist (2004). Methods of Analysis. Hops – 6A α and β-acids by spectrophotometry, Hops – 12 Hop Storage Index. 9th edition. The Society, St Paul, MN.

Biendl, M., Engelhard, B., Forster, A., Gahr, A., Lutz, A., Mitter, W., ... & Schönberger, C. (2015). Hops: their cultivation, composition and usage. Fachverlag Hans Carl.

Bocquet, L., Sahpaz, S., & Rivière, C. (2018). An overview of the antimicrobial properties of hop. In Natural Antimicrobial Agents (pp. 31-54). Springer, Cham.

Briant, L. (1905) The cold storage of hops, in The Hop and its Constituents – A Monograph on the Hop Plant (Chapman, A. C., Ed.)pp. 51–60, The Brewing Trade Review: London)

Briggs, D. (2004). Brewing science and practice (Woodhead Publishing Series in Food Science, Technology and Nutrition). Cambridge: Woodhead.

Briggs, D. E., Brookes, P. A., Stevens, R. B. C. A., & Boulton, C. A. (2004). *Brewing: science and practice*. Elsevier.

Brophy, J. (2016) Hop growing in the US: Where we've been, where we are now and what growers are doing to stand out. UW Extension, 7th Annual Hop Seminar Retrieved from https://buffalo.extension.wisc.edu/files/2011/01/2016-UW-Extension-Hop-Production-for-WI-Craft-Brew-Industry-To-Share.pdf Retrieved on 11/04/2020.

Brown D. (2013) Drying hops on a small scale. Retrieved from https://www.canr.msu.edu/news/drying_hops_on_a_small_scale Retrieved on 10/28/2020.

Brown D. (2014) Introduction to Hops. Retrieved from https://www.canr.msu.edu/uploads/236/71516/ipm_academy_2014_intro_to_hops.pdf Retrieved on 02/11/2020.

Canbaş, A., Erten, H., & Özşahin, F. (2001). The effects of storage temperature on the chemical composition of hop pellets. Process Biochemistry, 36(11), 1053-1058.

Carpenter, D. (2014), How to store hops Retrieved from https://beerandbrewing.com/how-to-store-hops/ Retrieved on 28/10/2020

Cattoor, K., Dresel, M., De Bock, L., Boussery, K., Van Bocxlaer, J., Remon, J. P., ... & Heyerick, A. (2013). Metabolism of hop-derived bitter acids. Journal of agricultural and food chemistry, 61(33), 7916-7924.

Clarke, B. J. (1986). Hop products. Journal of the Institute of Brewing, 92(2), 123-130.

Clark, S. M., Vaitheeswaran, V., Ambrose, S. J., Purves, R. W., & Page, J. E. (2013). Transcriptome analysis of bitter acid biosynthesis and precursor pathways in hop (*Humulus lupulus*). BMC plant biology, 13(1), 12.

De Cooman, L., Everaert, E., & De Keukeleire, D. (1998). Quantitative analysis of hop acids, essential oils and flavonoids as a clue to the identification of hop varieties. Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 9(3), 145-150.

De Keukeleire, D. (2000). Fundamentals of beer and hop chemistry. *Quimica nova*, *23*(1), 108-112.

Eyres, G. T., Marriott, P. J., & Dufour, J. P. (2007). Comparison of odor-active compounds in the spicy fraction of hop (*Humulus lupulus* L.) essential oil from four different varieties. Journal of agricultural and food chemistry, 55(15), 6252-6261.

Fandiño, M., Olmedo, J. L., Martínez, E. M., Valladares, J., Paredes, P., Rey, B. J., ... & Pereira, L. S. (2015). Assessing and modelling water use and the partition of evapotranspiration of irrigated hop (*Humulus lupulus*), and relations of transpiration with hops yield and alpha-acids. Industrial Crops and Products, 77, 204-217.

Fellows, P. J. (2017). Properties of food and principles of processing. Food Processing Technology, 3-200.

Ford, J. S., & Tait, A. (1924). NOTE ON THE VALUATION OF HOPS FOR ANTISEPTIC PROPERTIES. *Journal of the Institute of Brewing*, *30*(5), 426-429.

Forster, A. (2002). What happens to hop pellets during unexpected warm phases?. Brauwelt International, (1), 43-46.

Foster, R. T., & Nickerson, G. B. (1985). Changes in hop oil content and hoppiness potential (Sigma) during hop aging. Journal of the American Society of Brewing Chemists, 43(3), 127-135.

Garetz, M. (2005). Hop storage. Retrieved from https://www.morebeer.com/articles/storing_hops_properly#:~:text=For%20optimum%20preservation%20of%20hops,penetrates%20and%20causes%20some%20oxidation Retrieved on 10/262020

Gorst Valley Hops, (2018) Hop processing Retrieved from https://gorstvalleyhops.com/about/hops-processing/ Retrieved on 11/04/2020.

Green, C. P. (1997). Comparison of Tettnanger, Saaz, Hallertau and Fuggle hops grown in the USA, Australia and Europe. Journal of the Institute of Brewing, 103(4), 239-243.

Green, C. P. (1978). Kinetics of hop storage. Journal of the Institute of Brewing, 84(6), 312-314.

Grossman S, 2020 Hop farming spreads across the US, Retrieved from https://www.porchdrinking.com/articles/2020/03/09/hop-farming-spreads-across-us/ Retrieved on 11/02/2020

Hao, J., Speers, R. A., Fan, H., Deng, Y., & Dai, Z. (2020). A Review of Cyclic and Oxidative Bitter Derivatives of Alpha, Iso-α-and β-Hop Acids. Journal of the American Society of Brewing Chemists, 78(2), 89-102.

Hartley, R. D. (1967). Effect of oil content on the loss of alpha-acid from hops during storage. Journal of the Institute of Brewing, 73(6), 538-542.

Hieronymus, S. (2012). For the love of hops: The practical guide to aroma, bitterness and the culture of hops. Brewers publications.

Howard, G. A., & Slater, C. A. (1958). Effect of ripeness and drying of hops on the essential oil. *Journal of the Institute of Brewing*, *64*(3), 234-237.

Huang, Y., Tippmann, J., & Becker, T. (2013). Kinetic modeling of hop acids during wort boiling. International Journal of Bioscience, Biochemistry and Bioinformatics, 3(1), 47.

Hop Growers of America. (2019) Variety Snapshot. USA Hops retrieved from https://www.usahops.org/cabinet/USAHops_180948_VarietySnapshot_2019Web.pdf

Hughes, P. S., & Simpson, W. J. (1993). Production and composition of hop products. Technical quarterly-master brewers association of the Americas, 30, 146-146.

Kenny, S. T., & Zimmermann, C. E. (1986). Registration of 'Chinook'Hop. Crop Science, 26(1), 196-197.

Knez Hrnčič, M., Španinger, E., Košir, I. J., Knez, Ž., & Bren, U. (2019). Hop compounds: Extraction techniques, chemical analyses, antioxidative, antimicrobial, and anticarcinogenic effects. *Nutrients*, *11*(2), 257.

Krofta, K., Mikyška, A., & Hašková, D. (2008). Antioxidant characteristics of hops and hop products. Journal of the Institute of Brewing, 114(2), 160-166.

Kunze, W. (2004). Brewing Malting. Vlb, Berlin.

Lafontaine, S. R., & Shellhammer, T. H. (2018). Impact of static dry-hopping rate on the sensory and analytical profiles of beer. Journal of the Institute of Brewing, 124(4), 434-442.

Lafontaine, S., Varnum, S., Roland, A., Delpech, S., Dagan, L., Vollmer, D., ... & Shellhammer, T. (2019). Impact of harvest maturity on the aroma characteristics and chemistry of Cascade hops used for dry-hopping. Food chemistry, 278, 228-239.

Likens, S. T., Nickerson, G. B., & Zimmermann, C. E. (1970, May). An index of deterioration in hops (Humulus lupulus). In Proceedings. Annual meeting-American Society of Brewing Chemists (Vol. 28, No. 1, pp. 68-74). Taylor & Francis.

Malowicki, M. G., & Shellhammer, T. H. (2006). Factors affecting hop bitter acid isomerization kinetics in a model wort boiling system. Journal of the American Society of Brewing Chemists, 64(1), 29-32.

Malowicki, M. G., & Shellhammer, T. H. (2005). Isomerization and degradation kinetics of hop (Humulus lupulus) acids in a model wort-boiling system. Journal of agricultural and food chemistry, 53(11), 4434-4439.

Matsui, H., Inui, T., Oka, K., & Fukui, N. (2016). The influence of pruning and harvest timing on hop aroma, cone appearance, and yield. Food chemistry, 202, 15-22.

Menary, R. C., Williams, E. A., & Doe, P. E. (1983). ENZYMIC DEGRADATION OF α-ACIDS IN HOPS. Journal of the Institute of Brewing, 89(3), 200-203.

Mikyška, A., & Krofta, K. (2012). Assessment of changes in hop resins and polyphenols during long-term storage. Journal of the Institute of Brewing, 118(3), 269-279.

Moir, M. (2000). Hops—a millennium review. Journal of the American Society of Brewing Chemists, 58(4), 131-146.

Nance, M. R., & Setzer, W. N. (2011). Volatile components of aroma hops (*Humulus lupulus* L.) commonly used in beer brewing. Journal of Brewing and Distilling, 2(2), 16-22.

Nickerson, G. B., & Likens, S. T. (1979). Hop storage index. *Journal of the American Society of Brewing Chemists*, *37*(4), 184-187.

Nielsen, S. S. (Ed.). (2010). Food analysis (pp. 139-141). New York: Springer.

Ocvirk, M., Nečemer, M., & Košir, I. J. (2019). The determination of the geographic origins of hops (*Humulus lupulus* L.) by multi-elemental fingerprinting. Food chemistry, 277, 32-37.

Oladokun, O., Tarrega, A., James, S., Smart, K., Hort, J., & Cook, D. (2016). The impact of hop bitter acid and polyphenol profiles on the perceived bitterness of beer. Food chemistry, 205, 212-220.

Preedy, V. R. (Ed.). (2008). Beer in health and disease prevention. Retrieved from https://ebookcentral.proquest.com

Priest, M. A., Boersma, J. A., & Bronczyk, S. A. (1991). Effects of Aging on Hops and Liquid CO2 Hop Extracts. Journal of the American Society of Brewing Chemists, 49(3), 98-100.

Reeb-Whitaker, C. K., & Bonauto, D. K. (2014). Respiratory disease associated with occupational inhalation to hop (*Humulus lupulus*) during harvest and processing. Annals of Allergy, Asthma & Immunology, 113(5), 534-538.

Roberts, T. R. (2016). Hops. In Brewing Materials and Processes (pp. 47-75). Academic Press.

Rodolfi, M., Chiancone, B., Liberatore, C. M., Fabbri, A., Cirlini, M., & Ganino, T. (2019). Changes in chemical profile of Cascade hop cones according to the growing area. Journal of the Science of Food and Agriculture.

Rybáček, V. (Ed.). (2012). Hop production (Vol. 16). Elsevier.

Schindler, R., Sharrett, Z., Perri, M. J., & Lares, M. (2019). Quantification of α-Acids in Fresh Hops by Reverse-Phase High-Performance Liquid Chromatography. *ACS omega*, *4*(2), 3565-3570.

Schönberger, C. (2006). The processing of hops. In Brewing (pp. 123-148). Woodhead Publishing.

Sharp, D. C., Townsend, M. S., Qian, Y., & Shellhammer, T. H. (2014). Effect of harvest maturity on the chemical composition of Cascade and Willamette hops. Journal of the American Society of Brewing Chemists, 72(4), 231-238.

Skinner, R. N., Hildebrand, R. P., & Clarke, B. J. (1977). The effect of storage temperature on the stability of the alpha-acids content of baled hops. *Journal of the Institute of Brewing*, *83*(5), 290-294.

Skinner, RM. Kavanagh, TE & Clarke. BJ. Journal of the Institute of Brewing.

1979, 85, 7. 15

Srečec, S., Rezić, T., Šantek, B., & Marić, V. (2009). Hop pellets type 90: Influence of manufacture and storage on losses of α-acids. Acta alimentaria, 38(1), 141-147.

Srečec, S., Rezić, T., Šantek, B., & Marić, V. (2008). Influence of hops pellets age on α-acids utilization and organoleptic quality of beer. Agriculturae Conspectus Scientificus, 73(2), 103-107.

Steenackers, B., De Cooman, L., & De Vos, D. (2015). Chemical transformations of characteristic hop secondary metabolites in relation to beer properties and the brewing process: a review. Food Chemistry, 172, 742-756.

Taniguchi, Y., Matsukura, Y., Ozaki, H., Nishimura, K., & Shindo, K. (2013). Identification and quantification of the oxidation products derived from α-acids and β-acids during storage of hops (Humulus lupulus L.). Journal of agricultural and food chemistry, 61(12), 3121-3130.

Tedone, L., Staskova, L., Yan, D., Whittock, S., Shellie, R., & Koutoulis, A. (2020). Hop (Humulus lupulus L.) Volatiles Variation During Storage. Journal of the American Society of Brewing Chemists, 78(2), 114-125.

Van Holle, A., Van Landschoot, A., Roldán-Ruiz, I., Naudts, D., & De Keukeleire, D. (2017). The brewing value of Amarillo hops (*Humulus lupulus* L.) grown in northwestern USA: A preliminary study of terroir significance. Journal of the Institute of Brewing, 123(3), 312-318.

Vanevenhoven, K. P., Grogan, B. T., & Zeigler, N. S. (2019). U.S. Patent Application No. 16/129,776.

Verzele, M., & De Keukeleire, D. (2013). Chemistry and analysis of hop and beer bitter acids (Vol. 27). Elsevier.

Vollmer, D. M., Lafontaine, S. R., & Shellhammer, T. H. (2018). Aroma Extract Dilution Analysis of Beers Dry-Hopped with Cascade, Chinook, and Centennial. Journal of the American Society of Brewing Chemists, 76(3), 190-198.

Wain, J., Baker, C. D., Laws, D. R. J., & Wain, J. (1977). Deterioration of pelleted hop powders during long-term storage: Addendum: Commercial use of warm stored whole hops. Journal of the Institute of Brewing, 83(4), 235-240.

Wang, G., Tian, L., Aziz, N., Broun, P., Dai, X., He, J., ... & Dixon, R. A. (2008). Terpene biosynthesis in glandular trichomes of hop. Plant physiology, 148(3), 1254-1266.

Weber, K. A., Jangaard, N. O., & Foster, R. T. (1979). Effects of postharvest handling on quality and storage stability of cascade hops. Journal of the American Society of Brewing Chemists, 37(2), 58-60.

Williams, E. A., & Menary, R. C. (1988). Polyphenolic inhibitors of alpha-acid oxidase activity. Phytochemistry, 27(1), 35-39.

Williams, E. A. (1989). Inhibition of α-acid oxidase by polyphenolic compounds—a kinetic model. Phytochemistry, 28(5), 1327-1330.