Effect of Floor Germination Temperature on Dimethyl Sulphide Precursors Present in Malt and Sensory Characteristics of Beer

by

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Dedication

This thesis is dedicated to my Baba Kundanlal, parents Hemant and Smita, sibling Harshal, and cousins Manan, Chakshu, Kush, Tanmay, Hemu, Anu, Amrita, Harshita and Kashish. Achieving this qualification would have been a far-fetched dream without you all.

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Abstract

Dimethyl sulphide in beer tends to predominate overall flavour perceptions at very low threshold values. During malting, germination temperatures of steeped barley above 20°C induce formation of DMS precursors in malt (4), which ultimately evolve to DMS during brewing. To compare the impact of floor and pneumatic germination temperatures on the development of DMSP, two-row CDC Copeland barley was floor and pneumatically malted. Additionally, through a laboratory-scale floor malting protocol, barley was germinated at 10.0, 17.5 and 25.0°C to obtain green and kilned malt and fermented wort. Lastly, 30 L pilot brews were executed with floor and pneumatic malt to evaluate DMS threshold in beer made from floor malt. It was established that floor and pneumatic malting had a significantly different (p-value < 0.05) impact on DMSP levels generated. GC analysis detected the highest DMSP levels of 41.0 $\pm$ 10.3 µg/g and 10.8 $\pm$ 2.8 µg/g in pneumatic and floor malt, respectively. GC analysis of green malts germinated at 10.0, 17.5 and 25.0°C revealed no significant difference (p-value > 0.05) between DMSP levels generated at any germination temperature. Following kilning of these green malt samples at 63.0°C for 24.0 hours, GC results displayed no significant difference (p-value > 0.05) between DMSP levels obtained from samples. Furthermore, No DMSP was detected in fermented wort produced from lab floor malt. Results for sensory analysis for DMS (take-off threshold) in "floor malt" beers were inconclusive. Therefore, it was suggested that floor malt germination temperature can result in production of suitable malt which is suitable for brewers to use without resulting in off flavour development in final beer.

List of Abbreviations used

ADF	Apparent Degree of Fermentation
AE	Apparent Extract
ANOVA	Analysis of Variance
APA	American Pale Ale
ASBC	American Society for Testing and Materials
ASTM	American Society of Brewing Chemists
atm	Standard Atmosphere
CAR	Carboxen®
C_{max}	Point of Maximum Curvature
CMBTC	Canadian Malting Barley Technical Centre
DMS	Dimethyl Sulfide
DMSO	Dimethyl Sulfoxide
DMSP	Dimethyl Sulfide Precursors
DI H_2O	Deionized Water
DVB	Divinylbenzene
DW	Distilled Water
EMS	Ethyl Methyl Sulfide
FPD	Flame Photometric Detector
FID	Flame Ionization Detector
GC	Gas Chromatography
GC	Germinative Capacity
GE	Germinative Energy
HS	Headspace
H_2O_2	Hydrogen Peroxide
LOD	Limit of Detection
mL	Millilitre
MetSO	Methionine Sulfoxide
µg/L	Microgram per Litre
µL	Microlitre
µg/g	Microgram per Gram

n	Sample Size
NaOH	Sodium Hydroxide
n/d	Not Detected
ng/g	Nanogram per Gram
NIST	National Institute of Standards and Technology
OG	Original Gravity
^{o}P	Degrees Plato
PA	Polyacrylate
PDMS	Polydimethylsiloxane
SD	Standard Deviation
SMM	S-methylmethionine
^{o}SRM	Degrees Standard Reference Method
SPME	Solid Phase Microextraction
UBA	Universal Beer Agar
VOC	Volatile Organic Compounds
V/S	Versus
WS	Water Sensitivity
YEPD	Yeast Extract Peptone Dextrose

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1 Introduction

Beer is one of the oldest beverages widely produced and consumed throughout the world. The brewing process involves at a minimum the use of the raw materials water, malt, hops and yeast (1). All of these ingredients play key roles in imparting a characteristic flavour to beer.

Commercialization of beer increased significantly during the 19th century which led to expansion of malthouses, and floor malting was replaced by the pneumatic system, a batch process with controlled temperature and humidity variables. Lately with rising popularity of craft brewing, floor malting, has now been termed as craft malting. There are a few craft malt-houses around the country that have developed specific malting regimes. In floor malting, barley grains are germinated on a floor of the malthouse with no forced airflow (2). However, the consistency of malt and its influence on beer chemistry and flavor has not been determined substantially.

Dimethyl sulfide is a overcooked cabbage-like off flavor sometimes found in commercial beers ranging from 5-100 μg/L (3). Kavanaugh et al., (4) established that higher germination temperatures favour proteolysis in malt, which results in increased dimethyl sulfide formation in malt. Subsequently, White and Parsons (57) argued that barley germination yields in production of two DMSP's, one inactive and another active which form DMS in two different routes. The inactive precursor, s-methyl methionine (SMM) undergoes thermal degradation during kilning (> ~75^{o}C) while the active precursor known as dimethyl sulfoxide (DMSO) is metabolized by yeast to form DMS during wort fermentation.

This flavour defect can be eliminated from beer by modifying malt germination temperatures, increasing kilning temperature (>60^{o}C) and by increasing wort boiling vigour and boiling times. It could be expected that after implementation of these measures most of the DMS will evaporate. However, in some cases (e.g., a short wort boil) the finished product may still have detectable DMS.

Brewers select their malt fastidiously to obtain high yield extract and to avoid off-flavour in their final product. The presence of DMS in final beers can incur high energy costs (for removal) and result in decline in sales. Extensive wort boiling may not be feasible for all breweries. Meilgaard (6) reported that DMS thresholds vary with beer's chemical composition and preexisting DMS concentration.

Previous research has delineated a relationship between high germination temperature and DMSP generation. No information reporting the influence of uncontrolled germination temperature of floor malt on dimethyl sulfide precursors has been published. Hence, this thesis was directed to 1) analyze DMSP levels in floor malt samples germinated at three different temperatures and 2) evaluate DMS take-off threshold in beer made from floor malt.

2 Literature Review

2.1 Barley

Barley is classified under the *Poaceae* family, commonly known as the grass family and the genus Hordeum. Common barley is known as *Hordeum vulgare L.*

Barley grains suitable for malting are relatively homogeneous, distinct, bright yellow-white, plump, thin-hulled, medium hard and uniform in size (21). Whole barley grain subsists 65-68% starch, 10-17% protein, 4-9% β-glucan, 2-3% free lipids and 1.5-2.5% minerals (22, 23, 24, 19), and is used for animal feed, human consumption and malting.

Barley can be classified into several types, but with respect to malting, two types are popular; six-row and two-row types. The wild varieties are two-row barleys (8). With some difficulty, the grains of each variety can be distinguished by differences in the grain symmetry and the number of grains on each head (8, 9, 10). Six-row barley produces 20-60 grains whereas two-row barley generates 25-30 grains (11). For malting, high quality two-row barley is preferred because it results in malt with substantial extract, lighter colour, and lesser enzyme levels in comparison to the six-row variety (27). Hulled barley is normally chosen over hull-less barley for malting and brewing because the hull enhances the flavour of beer and aids as a filtering agent amid mashing (25). Furthermore, to be suitable for malting, it is crucial for barley to hydrate and germinate rapidly (28, 29).

2.2 Malting

A structured germination process followed by drying to accomplish physical as well as biochemical changes within the cereal grain is characterized as malting. Malting is comprised of:

1. Steeping – soaking of grains in water to increase their moisture up to at least 40%.
2. Germination – to hydrolyze the endosperm, synthesize enzymes and maintain embryo growth.
3. Kilning – drying of grain by blowing hot air and reduce the moisture content below 6% to ensure stability of grains (19).

2.2.1 Effect of Malting on Barley

Malting of barley can be visually distinguished by growth in coleoptile and rootlets. But on a microscopic level, malting is characterized by synthesis of hydrolytic enzymes, and alteration of starchy endosperm (31).

During malting, starchy endosperm of barley grain undergoes partial degradation along with other biochemical and physical changes that are referred to as modification (14, 15, 16). These modifications vary in each grain, as a result of which a range of degrees of modification is always found in malt, also known as heterogeneity (12).

2.2.1.1 Physical Changes

The primary indicator of malting is- acrospire or coleoptile growth. During germination, when the tip of acrospire extends halfway up the grain, its length is said to be ½; when the tip reaches grain's apex, its length is 1 and so on. Traditionally, green malt is kilned, and growth is terminated when most acrospires are 3/4th-1-of the grain's length (12). Refer to Figure 2.2.1.1 shown below.

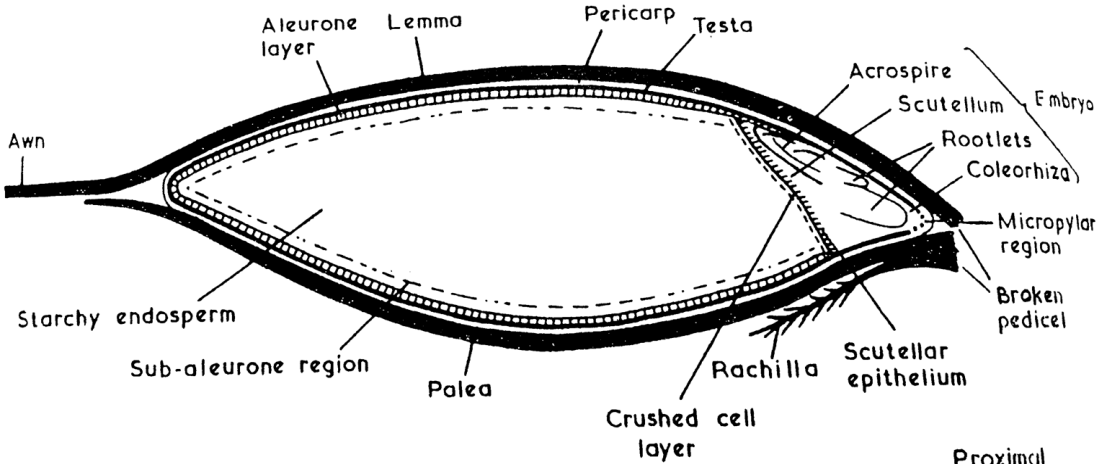


Figure 2.2.1.1: View of barley grain from ventral furrow side from Briggs 1981 (17)

The un-germinated seed has a tough surface, but with steeping, the moisture content increases, and the seed attains a softer texture, and is then specified as green malt. Conversion of barley to green malt is attributed to breakdown of β-glucan (32). When subjected to kilning, malt turns friable and brittle while the endosperm breaks up as flour (12).

2.2.1.2 Biochemical Changes

During steeping, moisture content increases and simultaneously, the metabolic rate and heat output increase as well. Steep water has dissolved oxygen, of which 50-70% is consumed by the embryo, and the rest by the aleurone layer of the grain (17). Barley endosperm endures a partial germination that leads to a cascade of enzymatic degradation of barley endosperm to increase the levels of bioactive compounds (19, 20). Seventy five percent of barley endosperm cell wall is composed of crosslinked (1-3), (1-4)-beta-D-glucans along with 20% arabinoxylans and protein.

These β-glucans and arabinoxylans influence the wort consistency and beer filtration (26). Barley modifications are brought about by enzymatic degradation of crosslinked (1-3), (1-4)-β-D-glucans and arabinoxylans. Enzymes that take part in this process are α-amylase, β-amylase, α-glucosidase, and limit dextrinase (19). The origin and direction of movement of these hydrolytic enzymes has been debated, especially during earlier stages of germination (36). However, several researchers presented strong evidence that barley aleurone cells can yield these enzymes (37, 38, 39). On the other hand, some scientists suggested that during germination, the scutellum could be an important source of these enzymes (40, 41, 42, 43).

Throughout endosperm modification, the cell wall matrix degrades to release starch granules (30). During kernel development, empty cells are crushed between expanding embryo and endosperm tissues to line the scutellar epithelium. This layer is abundant in β-glucan (44, 45, 18). These changes are associated with mobilization of cell energy reserves, the manufacture and release of hydrolytic enzymes and the uptake of low molecular weight materials from starchy endosperm and aleurone layers. These soluble

substances serve as nutrients for the growing embryo. The central embryo is degraded more slowly. Moisture is also lost by evaporation and incorporation into the products of hydrolysis (13,14). Starch is degraded but amylopectin is preferentially hydrolyzed by the enzymes (19).

Likewise, to solubilise hordein proteins and release starch granules, the matrix within endosperm should be broken down sufficiently. Breakdown of hordein proteins also supplies amino acids that serve as a nutrient for yeast during brewing (34, 35). Ultimately, extractable β-glucans are also reduced during kilning (33).

2.3 Dimethyl Sulphide (DMS)

DMS shown below in Figure 2.3, is the simplest thioether and nearly insoluble in water due to its non-polar structure.

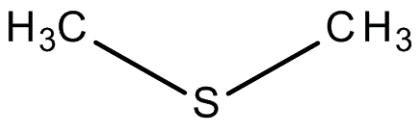


Figure 2.3: Structure of DMS

It is highly volatile and is a well-known flavour defect typically present in lagers but especially in commercially produced beers with short wort boiling times. It is usually detected in lagers because the malt is lightly kilned, and kilning is known to determine the extent of conversion of DMSP to DMS (49). On boiling, the produced DMS might be lost but can also be retained depending on the whirlpool rest time, boiling duration,

strain of yeast (since lager yeasts are functional at lower temperatures) and rate of evolution of CO_2 during fermentation (50). It has been established that different barley varieties yield different DMS levels and it has been observed that regional differences play a pivotal role in adding to the DMS levels. As well, malts made in the continent of Europe i.e., continental malts were found to have higher levels of DMSP present as compared to two or six-row pale ale malts, also known as, base malts and so they tend to generate more DMS (4, 5, 46, 47, 50).

Barley with high protein content and rapid modification tendencies were seen to possess increased DMS levels (113). With rise in storage time and temperature, DMS concentration in bottled wine also increased (132). Ferreira et al., (133) added that along with storage temperature, oxygen concentration also impacted DMS levels in wine. High levels of DMS were closely related to the presence of oxygen.

2.4 Dimethyl Sulfide Precursors (DMSP)

The two DMS precursors present in barley are s-methylmethionine (SMM) (Figure 2.5.1) and dimethyl sulfoxide (DMSO) (51) (Figure 2.5.2). Both originate from malt, but form DMS in different ways.

During modification, higher germination temperatures lead to the production of SMM in malt, and since SMM is a heat labile molecule, it releases DMS on kilning. The SMM is found with a ninhydrin reactive component; this combination is speculated to be the DMS precursor in green malt (52). This green malt precursor can only form free DMS by thermal decomposition. When malts are kilned at temperatures above 75^{o}C, majority

of SMM is degraded so free DMS is produced from DMSO during fermentation by yeast (52).

Scientists have also suggested that since SMM is produced during germination and controlling the kilning regime could lead to lower DMS levels in beers (as increasing the temperature would increase the breakdown of SMM) (4). Hudson (53) observed that during malt development, temperature is the dominant factor. High temperature in the summer accentuated dormancy while excessive moisture inhibited germination. After studying different craft malting house germination and kilning regimes, Griggs (54) noted that the technology of malting, barley variety, and airflow impact volatile compounds present in kilned malt.

2.5 Generation of Dimethyl Sulfide Precursors

Both precursors, s-methylmethionine and dimethyl sulfoxide are formed at different stages of malting and brewing.

2.5.1 S-methylmethionine (SMM)

SMM (Figure 2.5.1.1) as shown below is known as an inactive precursor and is produced during germination of malt (67). The level of SMM in green malt was a dominant factor in determining the level of SMM in kilned malt. In addition, L-methionine S-methyltransferase, also known as SMM synthetase was found to be present in barley and increased in activity during germination (68, 70, 74, 75, 76). The level of this enzyme in barley was correlated strongly to the levels of SMM in green malt and kilned malt and also with the level of DMSO in kilned malt.

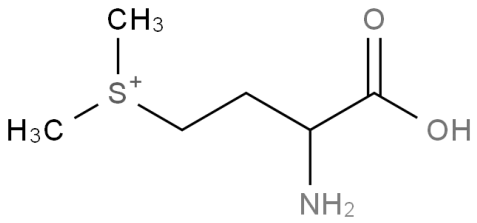


Figure 2.5.1.1: Structure of SMM

SMM synthetase converts S-adenosyl-L-methionine (SAM) and methionine to SMM during barley germination (Figure 2.5.1.2). Methionine is the acceptor of the methyl group, and SAM is the methyl group donor. With an increase in SMM, DMS increased, which resulted in increased wort protein. This correlation also suggested a probable connection between SMM synthesis and alterations in the barley protein-proteinase complex (133). During malt kilning and wort boiling, SMM is degraded into DMS and homoserine under high temperature (47, 67, 74).

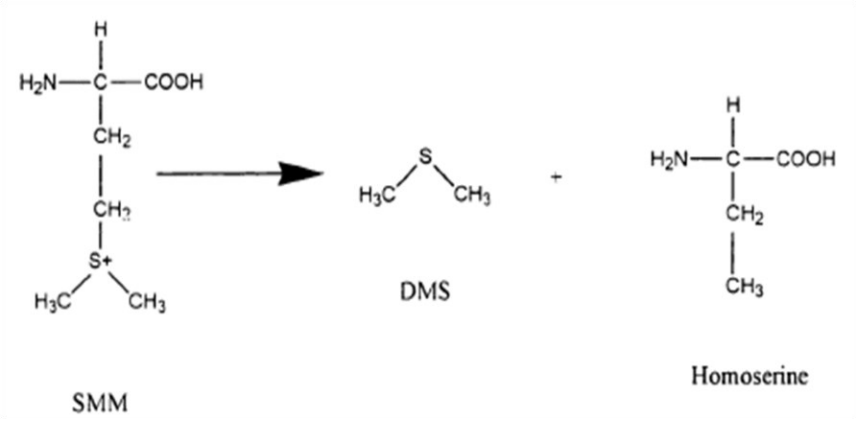


Figure 2.5.1.2: Degradation of SMM to form DMS and homoserine (56)

Conversion of SMM to DMS is a chemical heat decomposition (66). However, when barley was supplied with sulphur, increased levels of SMM in kilned malt were observed along with elevated malt diastatic power (134).

2.5.2 Dimethyl Sulfoxide (DMSO)

DMSO shown below (Figure 2.5.2.1) is a water-soluble, non-volatile compound, with a boiling point of 189°C that is formed by the oxidation of DMS that is released from SMM decomposition. DMSO in wort can be reduced to DMS during fermentation by the action of DMSO reductase present in brewing yeast as shown below (63) (Fig. 2.5.2.2).

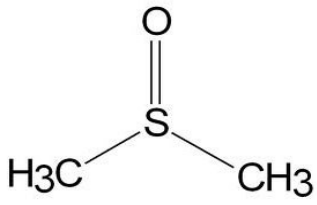


Figure 2.5.2.1: Structure of DMSO

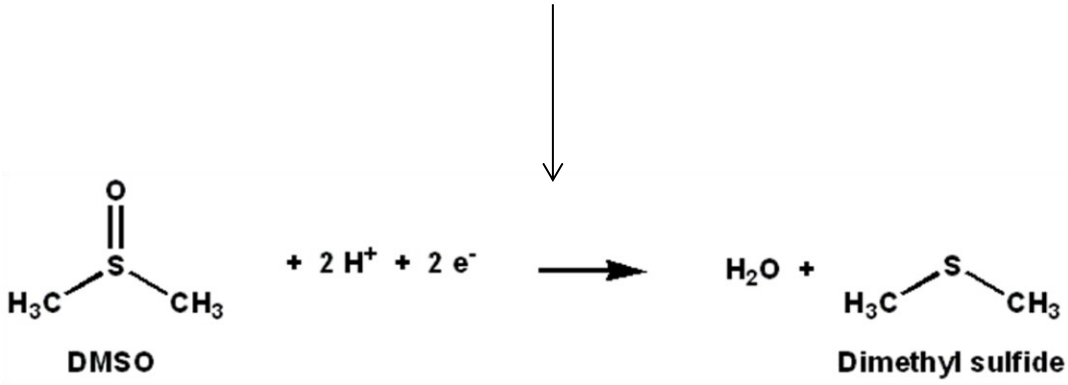


Figure 2.5.2.2: Reduction of DMSO by DMSO reductase to form DMS (63)

Beer spoilage bacteria have even higher DMSO reductase activities than yeast (73). Conversion of DMS to DMSO is a chemical oxidation reaction. Whereas conversion of DMSO to DMS is an enzyme-catalyzed reduction (66). The phenomenon of DMS oxidation to DMSO was observed in a sealed system under heat treatment (65). This

indicated that DMSO could be formed by oxidation of DMS, as SMM degrades during malt kilning. DMSO was present in malts at levels similar to those of SMM (69). Raw barley and green malt contain little DMSO (59). Annes et al., (65) reported that lager yeasts were able to reduce up to 21% of DMSO which was accompanied by significant DMS formation.

2.6 Principal Factors Influencing DMSP and DMS

2.6.1 Steeping and Germination Parameters

Pitz et al., (113) stated that high steep-out moistures, high sprinkling rates, high steeping and germination temperatures along with long germination times, abrasion and application of gibberellic acid, all augmented SMM production. Rootlets of germinated barley were rich in SMM. However, it was found that application of potassium bromate reduced SMM levels efficiently. When Dethier et al., (114) germinated barley at 14.0ºC with a moisture of 46% for six days instead of four, they observed a two-fold increase in DMSP levels. Interestingly when Yang and Schwartz increased the germination temperature from 16.0ºC to 20.0ºC they observed an increase in DMSP levels. However, when they increased the final grain moisture from 43% to 46% at the end of steeping, no significant increase ($p>0.05$) in DMSP was observed during germination (56).

2.6.2 Kilning

Kilning temperature was especially important in determining the final level of SMM and DMSO in malt (72). SMM degrades and DMSO appears at 70.0ºC. SMM could be

removed almost completely at 110.0°C, but DMSO increased to the highest level at higher temperatures. It was concluded that SMM synthetase activity played a major role in determining the level of SMM in green malt, which in turn influenced the levels of both SMM and DMSO in kilned malt (76, 68). SMM is degraded to DMS mainly at high temperatures during malt kilning and DMSO is reduced to DMS by yeast during beer fermentation (46, 64, 66, 77, 78).

During malt kilning, SMM is degraded into DMS and homoserine under high temperature (~70°C) (68). Kilning reduced the SMM content by an average of 50%. Increase in temperature from 78 to 85.0°C reduced SMM by 45% (68). Green malts with DMS levels ranging from 10.0-15.0 μg/g, when kilned were reported to have 1.0-10.0 μg/g of DMS on a wet weight basis (57), a substantial of DMS reduction by kilning.

2.6.3 pH

A rapid cleavage of DMSP occurs at pH 6 where an intramolecular substitution on part of DMSP's carboxyl group takes place to form DMS. While at low pH of 5.5 or 5.0, a nucleophilic substitution of sulphur group via water results in DMSP cleavage to form DMS. And DMSP cleavage at low pH requires more energy relative to high pH. However, this process is relatively slow. This necessitates adjustment of wort pH just before the end of boiling phase (104).

The enzyme SMM synthetase of germinating barley had an optimal activity at pH 6.0 and at 50.0°C. Moreover, this enzyme appeared to be partially resistant to kilning, which might impede complete removal of DMS from the malt during kilning (57). With an increase in the pH of wort the DMS level increased as well. It is noteworthy that lagers

have higher pH ranges of 4.2-4.75 while ales normally have lower pH ranges from 3.8-4.20, which can explain why lagers have a higher DMS concentration than ales (50).

2.6.4 Fermentation

During malting and beer processing, most of the DMS produced from SMM is lost to the atmosphere with the evacuation of gases during malt kilning, evaporation during wort boiling, or volatilization of CO_2 during beer fermentation. Only a very small part of DMS may remain in finished beer. Since DMS level is directly related to SMM, more attention is paid towards the level of malt SMM. The reduction in DMS may be accomplished by limiting the modification of malt and high kilning temperatures. Though such kilning regimes decrease SMM levels they often result in lowered diastatic power of malt and produce unacceptably high wort colours (113).

A change of DMSO to DMS by yeast may occur in wort or in different synthetic media. A high level of DMS in wort often masks the phenomenon of DMS production by yeast reduction of DMSO before fermentation and by a great loss of DMS, due to the promotion of the fermentation gases (CO_2). Frequently, only a slight increase of the DMS level in beer can be observed during later stages of fermentation and beer maturation (61, 62, 63, 65, 71). DMSO level in the medium does not affect the proportion reduced by yeast during fermentation; the amount of DMS formed will be significant if the DMSO content in the pitching wort is relatively high (78). This explains why it is a general consideration that only higher levels of DMSO in malt or in wort may be responsible for causing DMS problem in the finished beer. It was also found that overpitching of yeast and elevated fermentation temperature will promote more DMS volatilization at that start of fermentation (78).

Even though the quantities of DMS in un-pitched wort before fermentation and in beer after fermentation are similar, the knowledge that some free DMS will be lost during fermentation makes it clear that a significant proportion of the DMS in beer has been replaced from the reduction of DMSO by yeast. More than 65% of the DMS in beer is formed from DMSO under certain conditions (63, 65, 66). This occurs even if the DMS level in beer at the end of fermentation is lower than that at the beginning of fermentation. Unfortunately, this cannot be detected by an ordinary gas chromatography or gas liquid chromatography method if there is no net increase of DMS in beer during fermentation.

Several researchers have shown that the nature of DMS in the finished beer is produced only from SMM degradation (63). Naturally, DMSO seems to be more important than SMM in contribution to the DMS problems in beer. This is based on the fact that most free DMS in wort, formed through SMM degradation will be lost during fermentation and kettle boil. Only a small part of this DMS can survive into the finished beer. On the other hand, most of the DMS formed from DMSO reduction, especially during post-fermentation and beer maturation will persist in the finished beer.

2.6.5 Wort Boiling

On mashing green malt, no DMS evolved but on boiling this wort, 100.0 μg/L of DMS comparable to 1.0 μg/g of DMS in dry malt was obtained. Furthermore, heat alkaline treatment of green malt wort resulted in 500-1000 μg/L of DMS (57). This proved the presence of a heat labile DMSP, one that doesn't completely convert to DMS during wort boiling. Intensive boiling of wort will promote the elimination of DMS form the wort. In addition, rapid cooling of wort after boiling is necessary, since more SMM may

continue to be broken down into DMS and this DMS may not be lost. This is especially important when short boil times are used.

2.7 Types of Malting

The major difference between floor and pneumatic malting treatments is conduction of germination heat through the floor and radiation from the top of the grain bed and the airflow.

2.7.1 Floor Malting

The malting process exploits the natural tendency of barley to germinate, given the right conditions of moisture, temperature, and access to oxygen (53). In classical floor malting, the barley is spread out on floor in a layer 8.0-12.0 cm wide and 3.0-5.0" deep. Once the moisture content reduces to 14%, the grain is stored for six weeks to overcome seed dormancy. The grains are then first steeped in water two to three times for three days. Steeping increases the moisture of grains to around 46%. The grains are then transferred to a germination floor and are turned over by a rake for five days, while it is dried by natural air present in the room (2, 55). The batch size of traditional floor malt ranges from about 3-6 tonnes (12). However, at Horton Ridge, the batch capacity of floor malt was ~1 tonne. This germinated malt is known as green malt. The green malt is further kilned and toasted to obtain desirable colour and flavour specifications.

2.7.2 Pneumatic Malting

Pneumatic malting is often preferred to load, turn and transfer the the grains between the steep, germination and kilning unit operations in malting (12). It employs large industrial fans that blow air through, the germinating grain bed and to pass hot air through the malt being kilned. It is a batch process with greater size than floor malthouses (2). Conventionally a pneumatic system malts about 40 tonnes of malt at a time (12). Since pneumatic malting is a completely automated and controlled environment process, lower batch variation occurs in malt chemistry and flavour, resulting in the development of a more consistent product. Generally, grain beds range from 0.61m to 2.44m in depth, with increment in depth of grain bed, fans apply more pressure to increase airflow per unit cross sectional area to sustain a constant temperature between the top and bottom layers of the grain bed. Ideally, temperature differential should not exceed 1.0^{o}C but in practice, a difference of 4.0^{o}C is accepted (12).

2.8 Effect of Floor Malting on DMSP and DMS

It was observed that after malting, the concentration of DMS and DMSP was high in the roots of kilned malt, scutellum and acrospires of malt kernel (12). This led to the belief that biosynthesis might be responsible for high concentrations of DMSP and DMS in rootlets and acrospires of malt kernels. It was suggested that if malts with low DMSP levels are desired, biosynthesis should be kept to a minimum, consistent with adequate modification (4). The objective of controlling the modification is in accord with the general aims of maltsters as they primarily try to maximize enzyme production (i.e., modification) while minimizing the growth of roots and shoots since this growth

uses starch reserves of the endosperm, which reduces the yield. A further precaution in minimizing malt DMSP levels is to ensure thorough removal of rootlets.

Malt is the only contributor of DMS in wort. Kavanaugh et al., (51) found that DMS was absent from green malt and they suggested that kilning is a substantial processing step in actual formation of DMS, as the precursor SMM was thermally degraded to DMS. It was found that DMS content of kilned malt and presumably the level of inactive precursor in green malt can be kept low by using conditions which produce malts of low modification by using potassium bromate and applying low germination temperature (4, 46). Hyde and Brooks, (119) found that kilning of malt, grain bed depth, airflow and air temperature are all important in regulating final amounts of DMSP and DMS in malt. It was also found that on reducing the moisture content of the barley steep-out from 48% to 40%, decreasing the germination temperature from 20.0 to 12.0^0C, and relatively high initial kilning temperature (>65^0C) all decreased modification, and resulted in major reductions in malt DMSP levels.

However, barley grown with sulphur as fertilizer resulted in malts with higher free DMS than non-sulphured barley (134). Reduced airflow during germination lowered the DMSP level by almost 50% compared to normal airflow. Increased kilning times and temperatures invariably reduce DMSP levels (46). High steeping temperature causes seeds to uptake water faster and also induces rapid germination. A high germination temperature can result in a faster germination performance and growth but with hike of 1.0ºC from 20ºC, malting losses increased by 0.3% and the extract yields were lowered by 0.4%. Hence, Muller et al., (115) advised against germinating at higher temperatures.

White and Wainwright (49) found that the level of DMSP in British ale malts were significantly lower than lager malt probably due to differences in barley varieties, regional differences and malting procedures causing changes in characteristics such as colour, moisture, degree of modification, or amino nitrogen, as well as malting procedures. This was corroborated by Hysert et al., (46) who found that different barley varieties had different DMSP and DMS levels and regarded this as a consequence of barley varieties, malting companies, malt blends, crop years, as well as normal bin-to-bin and seasonal variations.

Expeditious malting brought about by rapid germination results in lowered levels of DMSP, mainly SMM (115). It was expected that DMSP induction would be accelerated with application of high germination temperature, but the impact of germination time was of much more crucial importance. Ales made from malt samples, germinated for a shorter time, had lowered DMSP levels. This would ultimately result in lowered energy costs by reducing kilning and boiling times (115).

2.9 Detection of DMSP

The accepted technological threshold of DMS in beer is 50-61 μg/L. While free DMS in light beers and pitching worts has been reported as about 20-80 μg/L and it is recommended that the total DMS content including the precursor should be < 100 μg/L (104).

White and Wainwright (49) developed a method to measure SMM that is heat labile with alkaline hydrolysis. ASBC developed a gas chromatographic method, Malt-14 to

measure both DMSO and SMM; both the precursors were exposed to high column temperatures to convert DMSP to DMS which was then measured by the GC equipped with a sulphur chemiluminescence detector (92). The difference between the total and free DMS gave the concentration of DMSP present in the malt sample. However, Hyde and Brookes (119) suggested that the alkaline hydrolysis method may not give an accurate measure of DMS levels in final beer as it measured DMS produced by both precursors collectively. Hysert et al., (116), developed a rapid, HS-GC method with an FPD detector that detected DMS directly from bottled beer.

Grigsby and Palamland (118) noted that DMS and sodium nitroprusside formed a pink complex, which could be detected spectrophotometrically. The absorbance of the pink complex corresponded to the amount of DMS. In their study, the majority of beers had DMS levels ranging from 40-70 µg/L while commercial lagers had DMS levels ranging from 30-160 µg/L. Dufor et al., (68) isolated SMM through HPLC on an ion exchange column, followed by a fluorescent complex formation between SMM and O-phthaldialdehyde. They observed that SMM concentration ranged from 4.6-19 µg/g of dry malt and reported the method as rapid, sensitive, and reproducible along with minimal sample preparation requirements.

Breeden et al., (117) found that conventional methods of addition of DMS in an aqueous solution, followed by autoclaving, evolved DMS as gas. They concluded that because of its high volatility and inability of being contained even in a parafilm sealed vessel held at -20.0^{o}C, it is difficult to develop an appropriate method for DMS detection.

Generally, SMM is measured by subtraction of free DMS from total DMS, but a study by Yang et al., (124) showed that there could be a variance of up to 300% in SMM levels when experimentation is executed in different laboratories. They also established that HPLC analysis for direct measurement of SMM took 105.0 min per sample and concluded that it is not feasible for everyday basis. They also reported that small quantities of DMSO are difficult to extract from aqueous wort samples because of its high solubility.

In order to avoid the time-consuming method of extraction of DMS via 25% potassium bromate solution, Ren et al., (126) introduced a microwave oven method to extract DMS in a shorter time period. Bottled barley was placed inside the microwave oven for 30.0 seconds with a treatment of 495W. Post extraction, a gas sample from this bottle was injected in GC-FPD set in sulphur mode to detect DMS.

The HS-GC method is preferred over others to measure DMSP since it's a rapid, highly sensitive and accurate method. There are many available detectors like flame photometric, flame ionization, and sulphur chemiluminescence. The Flame Ionization Detector (FID) is the most precise and satisfactory detector as the results are rapidly obtained with reproducibility. While, high variation is seen in the results obtained by FPD and sulphur chemiluminescence detector that is used in the ASBC official method for detection of DMSP, but its specific for sulphur compounds only and cost limits the use of this particular detector. Hence, FID is the widely used detector for detection of DMS and DMSP (64). Lately for sampling of VOCs, SPME fibres are preferred over HS because they desorb the VOCs fast enough to avoid the use of cryogenics along with smaller sample volume. SPME eliminates the preconcentration steps by directly

extracting analytes into a PDMS coated fibre which enhances selectivity of analysis, freedom to choose a stationary phase, reduction in matrix effects and interferences present in the liquid samples (85).

For a better extraction of non-polar and volatile flavour compounds, CAR-PDMS fibre is preferred over a PDMS 100 μm fibre or similar fibres (121). PDMS is a rugged fibre that can stand injector temperatures as high as 300^{o}C and is recommended for non-volatile flavour compounds. But when combined with carboxen; a porous activated carbon, retention capacity of the fibre increases because of increased potential of adsorption and distribution of analyte to the stationary phase. However, a CAR-PDMS fibre may have a poor repeatability (120).

Segueral et al., (122) modelled alkaline heat treatment for detection of SMM in wine through a 75.0 μm CAR-PDMS fibre. A DB-Wax column was used in combination with a split-less injector mode and helium as the carrier gas. Post heat treatment, free DMS concentration elevated from 36.9 to 2139 μg/L while DMSP levels decreased from 96 to 36.4 μg/L.

For the detection of volatile compounds like DMS at lower concentrations, static HS methods are not effective. Additionally, sulphur analytes react with the stainless-steel injection needle resulting in unreliable determination of DMS. For concentration of volatiles prior to GC injection purge and trap and cryo-focussing methods have been efficient but they are not economic methods as they require specially designed inlets with added operational costs which limits their use in majority of the labs. SPME has an advantage over all these methods because of minimal sample preparation, optimized

and automated sampling, and limits of detection in ng/L range (127). For non-polar volatiles, HS-SPME is recommended but analyte desorption from the polymer in the GC injector is slower than conventional solvent evaporation and leads the analyte peaks to have greater tailing. However, no solvent peak is displayed in chromatogram. Hence, in split-less injection mode, identification of volatiles in GC-MS chromatogram is simpler (129).

To detect volatile sulphur compounds, present in lower concentrations, a purge and trap method was utilized by Staffiso et al., (125) 10 mL of unfiltered wort and beer samples were used to measure DMS. Their GC-MS used a DB-5MS capillary column (60.0 m X 0.32 mm X 1.0 μm) comprised of 5% cross linked phenyl methyl siloxane. Lu et al., (123) employed a SPME-GC-FPD/FID to detect precursors of volatile organic sulphur compounds in algae induced black bloom. They used a 50/30 μm DVB-CAR-PDMS fibre.

For volatile analysis with SPME, sample vials were placed on heating blocks at 40.0, 50.0 and 60.0^{o}C for 1.0 hour, with SPME fibre inserted into HS (128). Furthermore, attainment of equilibrium results in maximum amount of analyte extraction. A proportional increase is observed in analytical signals with increase in extraction time. Incubation of sample at 60.0^{o}C with an extraction time of 10.0 min in a HS-SPME-GC-FID method resulted in improved extraction efficiency because of increased evaporation of the analyte concentration in HS. But samples cannot be over pressurised in SPME, so a temperature of 60.0^{o}C was recommended for this method (129).

Liu et al., (131) suggested preconditioning of fibre at 250.0ºC for 30.0 min before measurement of DMS/DMSP through a DVB-PDMS fibre (65 µm). Extraction of VOCs requires a thick coating while for semi-volatile compounds, a thin coating is more effective. Fibres covered with a thicker film have higher sensitivity because of greater extraction of analytes but such fibres take a longer time to achieve extraction equilibrium (135). So, while selecting a fibre for DMS analysis, the factors we considered were polarity and fibre thickness. Kremr et al., (136) compared a 50/30 µm DVB-CAR-PDMS, 75.0 µm CAR-PDMS and 85.0 µm PA fibres by exposing them to different temperatures of 30.0, 40.0, 50.0, 60.0, and 70.0ºC. They concluded that DVB-CAR-PDMS fibre provided the highest total signal and maximum number of peaks in chromatograms.

2.10 DMS Sensory Analysis

DMS has a low flavour threshold of 30-60 µL/L (46). Dialkyl sulphides such as DMS smell of cabbage or sweet corn and are characteristically present in lager beers at just under 1.0 flavour unit (6). Flavour unit is the equivalent of concentration of a flavour compound present in the beer divided by its threshold (160). There is no doubt that the malting conditions have a determining influence on the quantities of SMM synthesized, which are converted to DMS during beer processing. Only a very small part of DMS may remain in finished beer. A survey of Canadian beers showed that DMS had an estimated threshold of 68-69 µg/L (105). However, Grigsby and Palamland demonstrated a lower flavour threshold of DMS 25-30 µg/L (118), whereas Miracle et al., reported that commercial ales had an equal amount of DMS as did lagers despite the conventional belief that lagers have a high amount of DMS (127). Brown, Clapperton

and Meilgaard (103) reported a DMS threshold of 9-60 µg/L after doing a series of difference rating test. Scheuren (104) reported a DMS threshold range of 50-61 µg/L in beer by GC, which was universally accepted.

2.10.1 DMS Threshold in Beer made from Floor Malt

Thresholds of DMS might vary from one beer to another depending on the beer's chemical composition and upon the pre-existing concentration of DMS in those beers as well as seen in the section 2.10 above.

With increase in endogenous concentration of DMS, threshold concentration of DMS in beer also increases (6). According to Morrison (60) panels should have at least 12 members and preferably over 20 members as variations in threshold based on gender and genetic background occur. While Watts et al., (94) recommended that a sensory panel should have a minimum of 8-12 members. A tendency is seen for the variation between individuals to be smaller for small hydrophilic molecules of simple flavour and high threshold than for the larger hydrophobic molecules of more complex flavour and low threshold. An individual who shows low sensitivity to one compound can show average or high sensitivity to other compounds (58). According to this statement, high variability should be expected with low threshold value as DMS is a non-polar, hydrophobic compound.

Hegarty et al., (84) found that higher levels of 2-phenylethanol suppressed the perception of DMS in final beer and additionally, reported that flavour of sour beers and bacterial contamination in beer might impede measurement of DMS threshold.

While, Griggs (54) observed that barley variety selection and airflow influenced volatile compounds found in kilned base malt. Lack of forced air movement through the grain bed causes a localized blanket of CO_2 to form around every kernel of grain, which slows respiration and modification, and gives the malt a rich aroma and flavour (59). Contaminating spoilage bacteria like the *Enterobacteriacease* or *Obesumbacteriaceae* have even higher DMSO reductase activity than yeast (79).

Thus, DMS formed through DMSO reduction may constitute the main portion remaining in beer and cause off-flavour problems form higher DMSO levels in wort (77). There are several ASTM methods available for estimation of a group sensory threshold. One of the most common and widely used standard method is ASTM E679 (86) that is widely used to calculate an individual best estimate threshold as it accounts for the variability among people and is a rapid method, it calculates thresholds on the basis of correct/incorrect responses given by each panelist. A group threshold is further calculated by doing a geometrical average of the individual best estimate threshold of all the panelists. Similarly, the ASBC Sensory Analysis-9 method for threshold evaluation of added substances employs a triangle test (six sets) to determine a difference threshold. This analysis first identifies individual best estimate threshold by calculating the geometric mean of the highest concentration missed by the participant and further group thresholds are derived from the individual thresholds. These tests require panels of 5-15 members but in order to attain greater validity, a 20-30-member panel is recommended (160).

A group threshold for DMS needs to be calculated for beer made with floor malt, since any change in malt characteristics directly affects the flavour, stability and other

characteristics of a finished beer which, in turn, affects flavour threshold of DMS in that particular beer. Marin et al., (96) developed a method called "Threshold approximation using the point of maximum curvature of a logistic curve." The model is depicted in Figure 2.10.1 below.

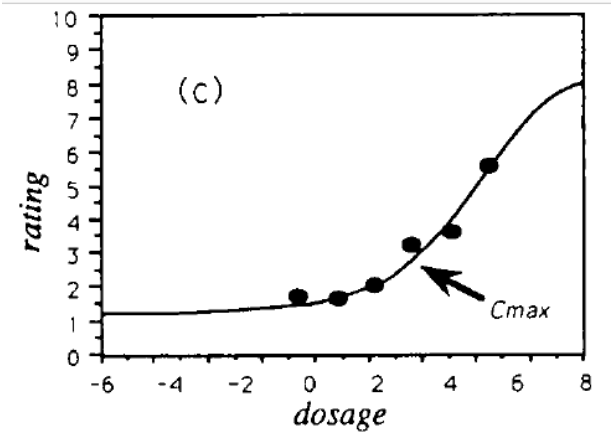


Figure 2.10.1: Dose response curve by *Marin et al., 1991*

where C_{max} is the take-off threshold.

The methods, E679 and ASBC Sensory analysis-9 use quantitative values of threshold to differentiate individuals by their sensitivity. However, the method by *Marin et al.,* (96) mentioned above, is more appropriate for this study because with the use of logistic curve to fit the data points to give a take-off threshold (C_{max}), it also links behavioural response of an individual (or population) to chemical changes in the product (beer). Which implies that along with a quantitative response, a qualitative response (behavioural response) is also obtained. This concentration of DMS at which response of individuals starts to change rapidly with increasing concentrations can also be used to characterize the subject and stimuli, both.

3 Objectives

The principal objective of this thesis is to characterize the floor malting germination process that affects the DMSP formation in malt as compared to pneumatic malting. The hypotheses studied are:

1) High and uncontrolled germination temperatures in a modern-day floor malt house lead to increased production of DMS precursors in malt, ultimately resulting in beer with high DMS levels.
2) The "take-off threshold" for DMS in floor malt beer will be lower than pneumatic malt beer.

To examine these hypotheses, a lab-scale floor malting procedure with three germination temperatures (10.0, 17.5, or 25.0°C) was devised, and batches of green and kilned malt were obtained. Small-scale fermentations as per ASBC method Yeast-14 assay were carried out on this malt to obtain fermented wort samples. Throughout the fermentation, apparent extract, colour, and turbidity readings were taken as per ASBC methods 10(A) and 2(B).

To evaluate the amount of DMSP generated and lost during malting to brewing, a Headspace Solid Phase Microextraction Gas Chromatography Flame Ionization Detector (HS-SPME-GC-FID) Analysis was developed and performed on malt germinated at different temperatures and their respective wort samples.

Pilot brews (30 L) were executed with larger amounts of malt. A sensory panel was set up and trained to estimate DMS flavour take-off threshold in those beers.

4 Experimental Design

The experimental design of this thesis is composed of both, analytical and sensory modules. Preliminary tests involved germination capacity, germination energy and water sensitivity of barley procured. The first section concenters on detection of DMS precursors in malt and fermented wort through a HS-SPME-GC-FID analysis. A lab-scale floor malting protocol was designed to germinate barley at 10.0, 17.5 and 25.0^{o}C and inspect which germination temperature induced higher production of DMSP. Simultaneously, commercially available floor and pneumatic malt were analyzed to draw comparative values of DMSP. The lab kilned malt was mashed and fermented as per ASBC Yeast-14 analysis, to obtain fermented wort, which was further analyzed through the same method. Additionally, commercially available beers made with pneumatic malt were also assessed for DMSP levels to draw a comparison.

The lab floor malt sampling scheme is illustrated in Figure 4.1. (next page) for each germination temperature (10.0, 17.5 or 25.0^{o}C), three types of samples were obtained. First, green malt samples were procured right after germination, labelled and stored at -30.0^{o}C. Second, the rest of germinated barley was kilned at 63.0^{o}C to obtain a pale kilned malt. Third, fermented wort was prepared from a portion of kilned malt through Yeast-14 assay by ASBC; this wort was stored in well labelled 50mL Falcon tubes (Fisher Scientific, Thermo Fisher Scientific, Waltham, MA) at -78.0^{o}C.

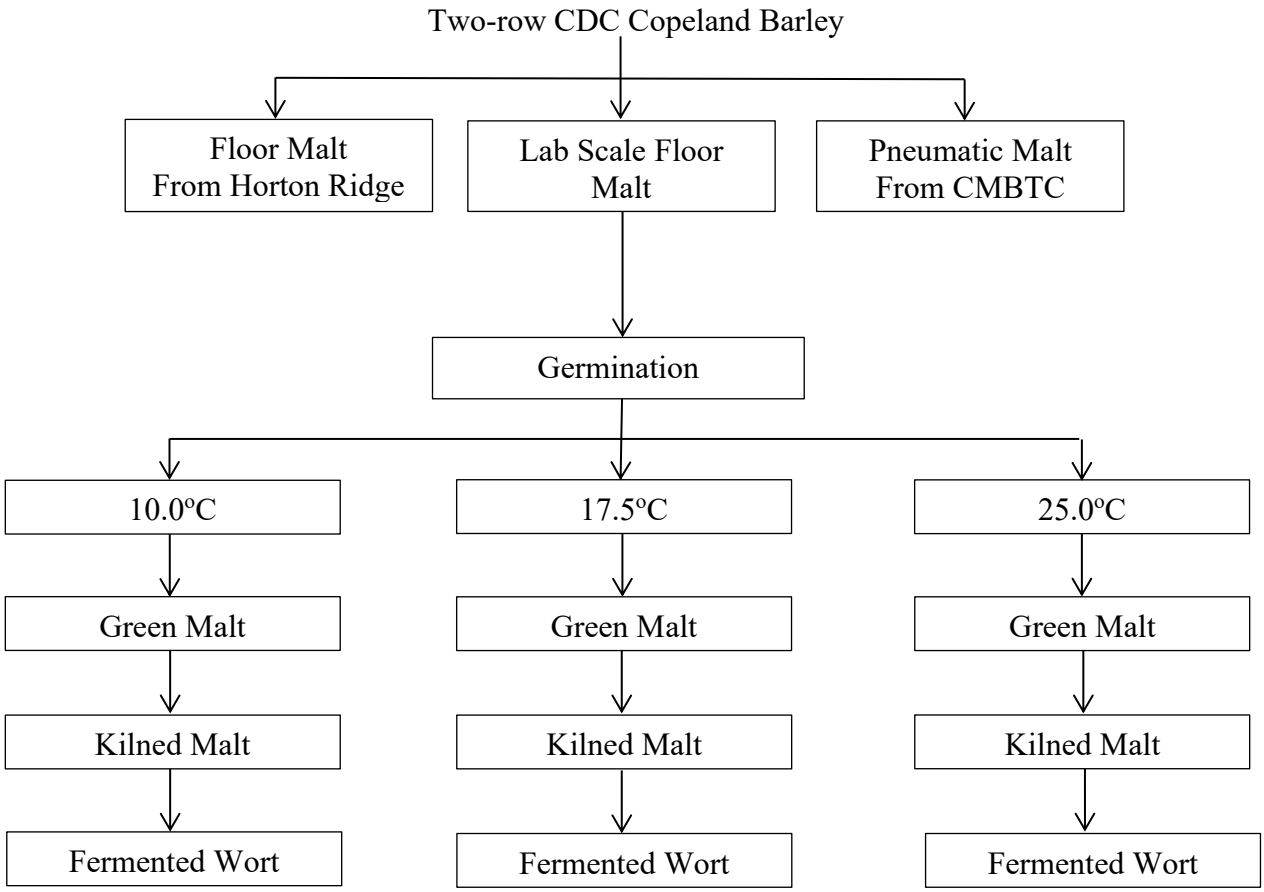


Figure 4.1: Malt process flow and sampling scheme for DMS precursor detection

The subsequent section of this thesis is aimed at evaluating DMS take-off threshold in beers made from floor malt. Thirty-liter pilot beers were brewed with both Horton Ridge floor malt and Canadian Malting Barley Technical Centre (CMBTC) pneumatic malt. These brews were fermented, cold crashed and carbonated. Meanwhile, a panel of 10 members was trained to identify flavour of DMS in commercially available beers. To test if take-off threshold for DMS in floor malt beer will be lower than pneumatic malt beer; Triangle tests were performed on both beers followed by the threshold tests. For threshold tests, floor malt beer was spiked with food grade DMS in concentrations ranging from 30-150 µg/L and presented to the participants in randomized order in a proper sensory setting. Every participant was asked to rate spiked samples from 0-8

with respect to their difference from the blank beer with no additional DMS. A framework of the experimental design is delineated in Figure 4.2 below.

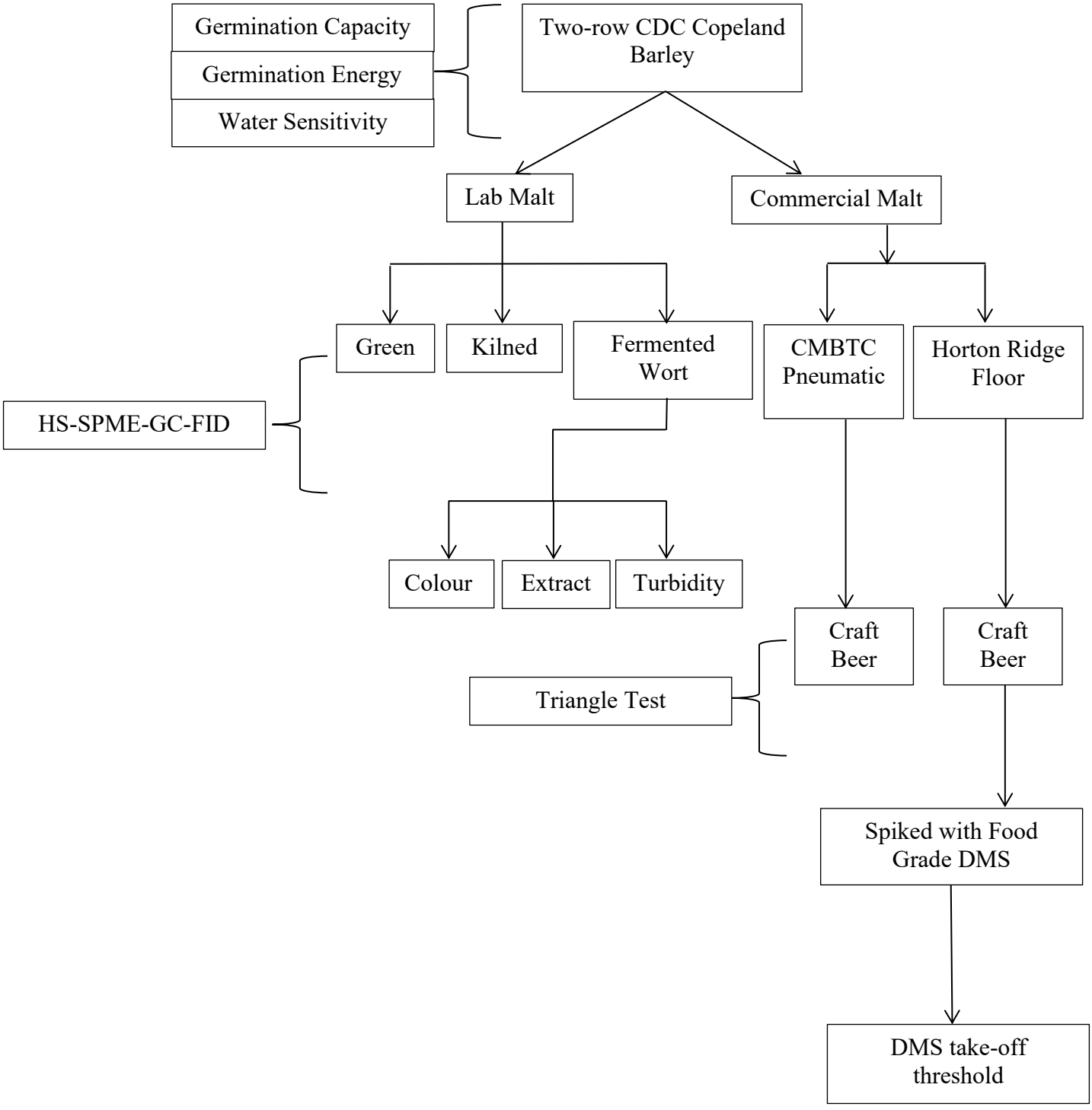


Figure 4.2: The experimental design framework

5 Materials and Methods

5.1 Materials

5.1.1 Barley

Two-row CDC Copeland malting barley harvested in 2017 was from a single source farm in Saskatchewan.

5.1.2 Lab-Scale Floor Malting

A lab scale floor malting method was devised after consulting a local floor malt house for their malting protocol, steeping, germination and kilning regime.

5.1.2.1 Steeping

The steeping used comprised a cycle of overnight soaks lasting for 17 hours (x three), these soaks are apportioned by air rests lasting for eight hours (x two). A 1.5 Kg lot of barley was weighed and transferred to tall cylindrical beakers. The barley was first washed with cold water to get rid of soil particles. Then, running tap water at a temperature of 12-15.0^{o}C was poured on the barley, the beaker was covered with aluminum foil and kept at room temperature for steeping. After the soak and air rest cycles were over, wet basis moisture was calculated as per the standard formula for each batch of steeped barley. Moisture ranged from 41-48%.

5.1.2.2 Germination

After recording frequent germination temperatures at the malt house throughout the summer of 2018, three germination temperatures; 10.0, 17.5 and 25.0°C were selected for lab-scale germination set-up. After steeping, barley was further transferred onto borosil baking trays. These trays were placed in incubators (MIR-153, Sanyo Scientific, JPN) at temperatures 10.0, 17.5 and 25.0°C for germination. At higher temperatures barley germinated for four days and at 10.0°C it germinated for five days. To measure progress of germination, 20 kernels of barley from each germinating batch were taken and their acrospires length was measured, once the length of 75% acrospires was around $3/4^{th}$ quarter of the kernel, the germination was concluded. The malt obtained right after germination was divided into two parts, green malt and kilned malt. The green malt was stored in ziploc pouches, labelled and frozen at -30.0°C to prevent any chemical changes.

5.1.2.3 Kilning

With reference to the local floor malthouse's kilning regime, a lab-scale kilning system with a food dehydrator (Iona ED-700, Excalibur, Sacramento, CA) as kiln was devised. The malthouse applied a temperature range of 50-70.0°C to drop the moisture of malt to 10% and then 80.0°C to reduce to a moisture of 5%. Corresponding to which and considering the temperature limitations of the food dehydrator, germinated barley was first dried at 50.0°C for four hours, and then the temperature was increased to 63.0°C for the next 24 hours. The moisture of malt was checked, and the kilning continued until the moisture dropped to or below 5%. The kilned malt was stored in labelled ziploc bags and placed in a cool and dry area.

5.1.3 Pneumatic Malt

Canadian Malting Barley Technical Centre (CMBTC, MB), processed the same two-row CDC Copeland malting barley to make pneumatic malt. The barley was steeped in tap water at 12-15.0°C for three days with periodic air-rests. Barley was then germinated at 14.0°C for 96 hours. For kilning, the grains were kilned at 50.0°C until the moisture dropped to 10%, 60.0°C for three hours, 70.0°C for one hour, and finally at 80.0°C until the moisture dropped to 5%.

5.1.4 Floor Malt

Horton Ridge, a local floor malthouse malted the same two-row CDC Copeland Barley to supply floor malt. The average germination temperature throughout the summer was 19.6°C, while the highest temperature recorded was 26.0°C. The germination continued for 5 days along with periodic raking done manually. The kilning regime was the same as mentioned in section 5.1.3 above.

5.1.5 Yeast Strain

Saccharomyces pastorianus strain SMA was procured from White Laboratories via CMBTC, Winnipeg, MB.

5.1.6 Yeast Culture

Yeast cells were propagated on Universal Beer Agar (Sigma Aldrich, St. Louis, MO) slants and plates. The yeast from these plates and slants was further inoculated in Yeast

Peptone Dextrose Broth in a C24 Incubator Shaker (New Brunswick Scientific, Edison, NJ) at 100 revolutions per minute for 24 hours and 30.0ºC. Yeast was harvested by centrifugation at 3000 x g for 3.0 min (x three).

5.1.7 Wort Preparation by Congress Mash

Wort was prepared from all malts processed in the laboratory. ASBC method Malt-4 for extraction of malt was conformed to (88). Treatments of 55.0 g of each malt batch were ground in a DLFU laboratory disc mill, at setting 2.0 (Bühler Universal, CHE) and then weighed to 50.0 g by removing extra 5.0 g. Simultaneously, Congress mash program was selected on the mash bath (Industrial Equipment and Control Pvt. Ltd., Melbourne, AUS). 200.0 mL of distilled water was poured into each mash beaker of the mash bath and let to heat up to 45.0ºC. Ground malt was added to the hot water immediately and this mixture was mixed with a glass rod to avoid formation of lumps. This mixture was stirred with magnetic stir bars throughout the mashing process. The mash beakers were covered with watch-glass in order to prevent evaporation. The mashing program maintains the temperature of mash at 45.0ºC for 30.0 min and then increases mash temperature by 1.0ºC/min until 70.0ºC is reached. At 70.0ºC, 100.0 mL of mashing-in water was added to each mash beaker and held at 70.0ºC for 1 hour. To cool down, the mash program drops mash bath temperature to room temperature in 15.0 min. The mash is filtered into a 500.0 mL Erlenmeyer flask through a funnel lined with a Whatman filter paper (30.2 cm, Whatman, GE Healthcare Life Sciences, Marlborough, MA). The filtered wort was autoclaved at 121ºC at an atmospheric pressure of 1.02 atm to prevent microbial contamination and cooled down to room temperature and stored in the refrigerator for subsequent experiments.

5.1.8 Lab Fermentations

The wort obtained from method 5.1.7 above was utilized for this process. A test tube fermentation was performed for each malt extract batch prepared in the lab, to brew small amounts of beer under controlled conditions. ASBC method Yeast-14, Miniature Fermentation Assay (89) was referred to for this experiment. Yeast was harvested and pitched in 450.0 mL of wort. This wort was pipetted into 30 fermentation test tubes (15 mL in each tube). The fermentation continued for 72.0 hours, during which turbidity and density readings were collected three times per day. For the last reading, colour measurement was also taken as per method Beer-10, (A) (99). Three tubes containing beer for each set were filtered by VWR paper 5 (9.0 cm, VWR, Missouri City, PA) and transferred to labelled centrifuge tubes and frozen at -78.0ºC to avoid any chemical changes throughout the storage. This fermented wort samples were tested for presence of DMSP by gas chromatography method as described in section 5.2.2.

5.1.9 Pilot Brews

Pilot brews were executed at 2 Crows Brewing Co., Halifax (NS). A lightly hopped American pale ale was brewed with both, pneumatic and floor malt, with 12-15 international bitterness units. Two lots of 10.0kg of floor and pneumatic malt were used to brew. It was a single infusion mash, mashed in at 66.7ºC for 60.0 min and mashed out at 75.6ºC for 10.0 min. The obtained wort was then filtered and transferred to kettle, to boil for 90.0 min. In the last five min of boil time, 30.0g of Azacca hops (Brew Culture Inc., Bracebridge, ON) were added. While 56.0g and 20.0g of Huell melon and Citra hops (Hops Connect-Hop Trading Company Ltd., Centralia, ON) were added post boil, respectively.

Original gravity (OG) readings were taken before pitching the yeast. The proprietary ale yeast of 2 Crows Brewing Co., Halifax, NS was used. The wort extract of floor APA brewed in duplicates was 11.43 and 11.91°P. The wort extract of pneumatic APA brewed in duplicates was 13.57 and 12.39°P. The wort was cooled to 18.0°C and transferred into a keg, where 200.0mL of ale yeast was added. The kegs were capped and left to ferment at 18.3°C for seven days. Post fermentation, kegs were cold crashed to get rid of yeast and hop slurry, this beer was further transferred to new kegs and then force carbonated at 1.70atm. Approximately, 30.0L of floor and pneumatic APA was made in each batch and these brews were conducted twice. The beer kegs were stored at 4.0°C. These beers were used to undertake a comparative sensory study for both malts.

5.2 Methods

5.2.1 Germination Quality

To verify germinative quality of barley used for our studies. These tests were conducted as per the ASBC method Barley-3(B and 3C) in triplicate (90, 91). Double filter paper (9.0-cm, VWR-5, Missouri City, PA) was placed in the bottom of each of four petri plates labelled as A, B, C and D and 100 kernels of barley were put in each plate. In plates A and B, 4.0 mL of water was added while in plates C and D, 8.0 mL of water was added. All plates were stored in ziploc bags and incubated in a cabinet at room temperature. The chitted (growth of acrospire on the tip of the grain) kernels were removed after 24.0, 48.0 and 72.0 hours.

5.2.1.1 Germinative Capacity

An aliquot of 100.0 mL of hydrogen peroxide (0.75%) solution was added to 100 kernels of barley placed in a 150.0 mL beaker covered with watch glass. After 48.0 hours of incubation, the chitted (growth of acrospires) kernels were removed and H_2O_2 solution was drained. A second aliquot of 100.0 mL of freshly prepared (0.75%) H_2O_2 solution was added to left over kernels and after 98.0 hours, the chitted kernels were counted again and applied to the formula provided in the standard method to give the germinative capacity of the barley (90).

5.2.1.2 Germinative Energy

Germinative energy is a measure of vigour with which barley kernels germinate. 100 kernels of barley were germinated in water only in plates A and B for 72.0 hours. After 72 hours, chitted kernels were counted to calculate the germinative energy to closest percent (91).

5.2.1.3 Water Sensitivity

Water sensitivity refers to the ability of the grains to cope with water stress, i.e., water abundance or deficiency. Number of kernels germinated after 72.0 hours of incubation in water in all four plates A, B, C and D were counted, water sensitivity was calculated and reported to closest percent (91).

5.2.2 DMSP Detection by HS-SPME-GC-FID

DMS detection methods are a combination of the standard ASBC method Malt-14 (92) and one of Morisaki et al., (93) and is illustrated in Figure 5.2.2 below. It is a Headspace Solid Phase Microextraction Gas Chromatographic method with Flame Ionization Detector (HS-SPME-GC-FID). It corresponds to the amount of DMSP present in malt (seeds) and wort samples on the basis of difference between total and free DMS concentrations obtained before and after alkaline heat treatment. Six standards were prepared with analytical grade DMS ($\geq$ 98% GC, Sigma Aldrich, Oakville, ON) concentrations of 0.0, 2.0, 4.0, 6.0 and 8.0, 12.0 and 16.0 µg/L with 8.0 mL of distilled water in glass vials. Solutions were mixed immediately, and vials were sealed tightly. Each vial was placed on a digital dry bath at 40.0ºC for 30.0 min, with a 1.0 cm DVB-CAR-PDMS fibre (Supelco, Sigma Aldrich, Oakville, ON) inserted into its headspace. A digital dry bath is a heating block filled with metal chips to incubate the samples. A calibration curve for DMS was prepared by this data (92). The calibration was performed periodically and resulted in a $r^2 = 0.96$. For the actual samples two types of vials were made, free DMS and total DMS. Fibre was preconditioned immediately prior to use at 240ºC for 37.0 min. It was inserted in the injection port of GC-FID (Varian 450 GC, Bruker Corporation, Billerica, MA) system equipped with a Zebron-Wax column (30 m x 0.25 mm x 1.0 µm, Phenomenex, Torrance, CA).

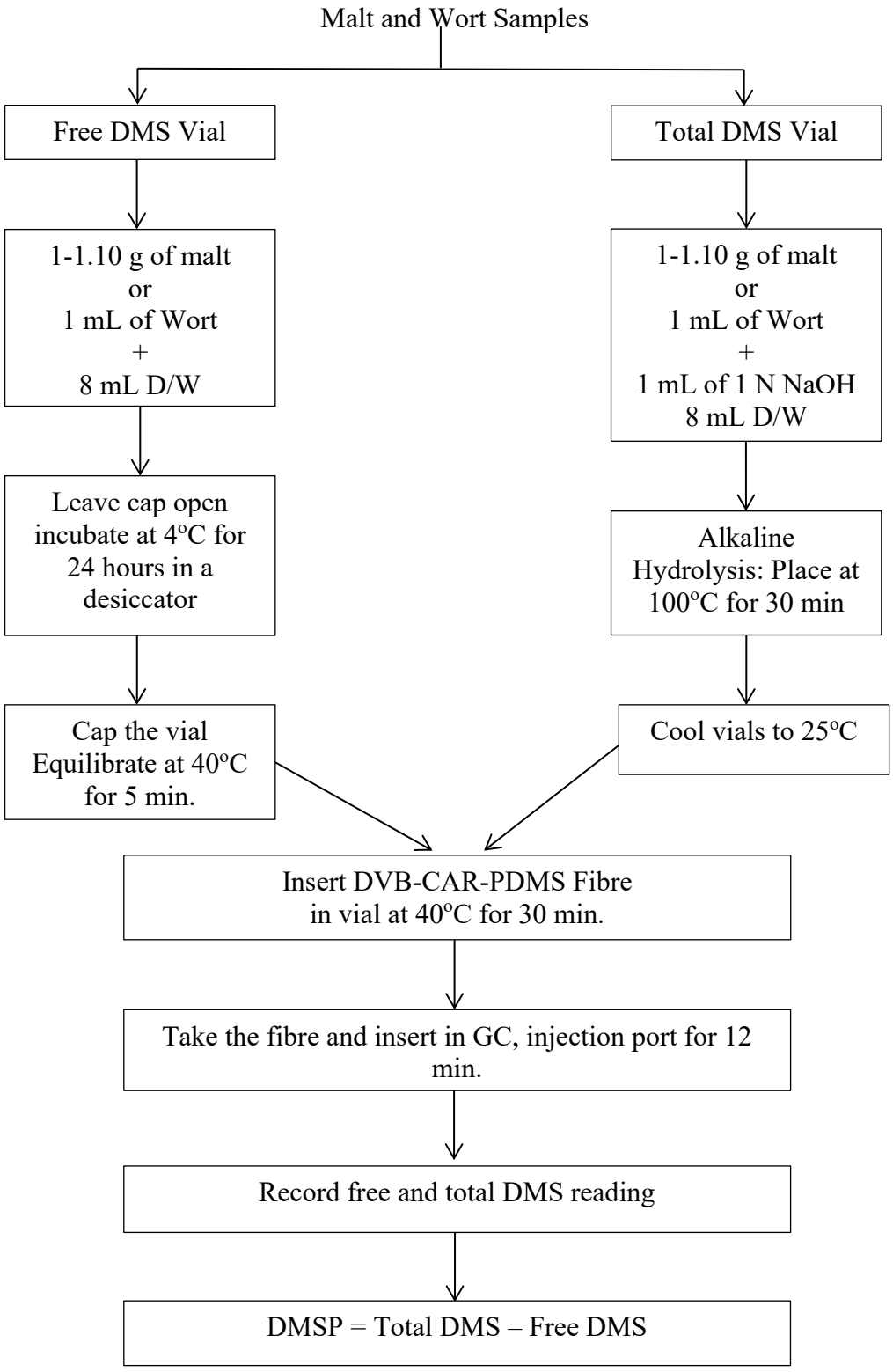


Figure 5.2.2: Representation of Dimethyl Sulfide precursor detection by HS-SPME-GC-FID

The free DMS vials had 25-26 seeds of malt corresponding to 1-1.05g, soaked in 8.0mL of distilled water for 24.0 hours at 4.0°C was added in a glass vial (22mL, PerkinElmer, Waltham, MA). Prepared in triplicate. The vial was kept open during the soaking period in a desiccator. Thereafter, it was sealed tightly with a Teflon septum and a screw top seal (National Scientific, Rockwood, TN). These vials were placed in a digital dry bath (Fisherbrand Isotemp, Fischer Scientific, Ottawa, ON) at 40.0°C, with fibre was inserted into the headspace for 30.0 min. Equilibration time was five min at 40.0°C, without the fibre.

For total DMS vials, another batch of 25-26 seeds corresponding to 1-1.05g were put in a vial, 1.0mL of 1N NaOH, and 8.0mL of distilled water were added to the vial. This vial was sealed and boiled in dry bath at 100.0°C for 30.0 min. These samples were alkaline hydrolyzed to release the DMS from DMSP present in the seeds and were prepared in triplicate. The alkaline heat-treated seeds were then cooled, and the volatiles were collected at 40.0°C using a 1.0cm DVB-CAR-PDMS fibre (Supelco, Sigma Aldrich, Oakville, ON).

All the samples were coded and randomized in order to avoid any form of bias.

The column temperature was programmed as follows: 40.0°C for 5.0 min followed by an increase of 5.0°C/ min to 200.0°C. Carrier gas helium was delivered at 86.1 kPa with a rate of 1.0 mL/min. Split ratio of 1:10, injection with a sampling time of 1.0 min was used. Fibre was held for 12.0 min in injection port to completely remove any compounds from matrix. Temperature of the injector and interface should be 240.0°C (93).

5.2.3 Retention Index of DMS

A 150 µL microinjection needle was used to inject analytical grade hexane and decane (≥ 98% GC, Sigma Aldrich, Oakville, ON) into filter paper, which was then placed in a 6 L Erlenmeyer flask. The DVB-CAR-PDMS fibre was then inserted in the headspace of flask for 30.0 min and later injected in the GC. The run was continued for 12.0 min. Refer to figure 5.2.3 shown below.

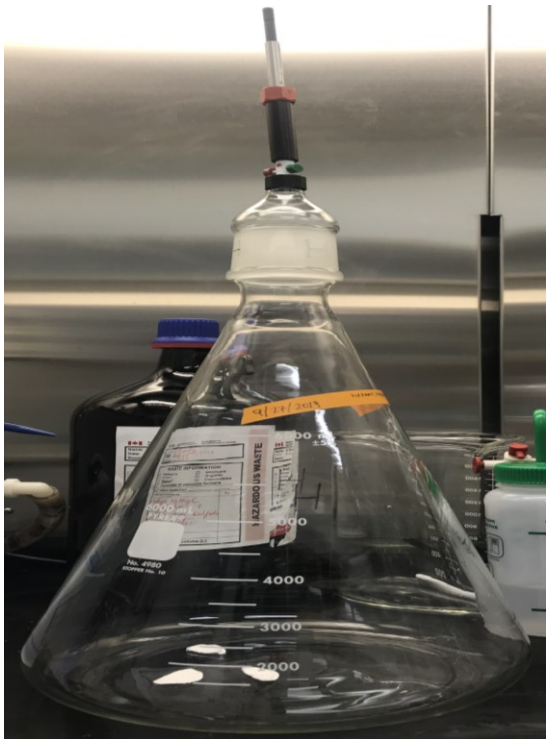


Figure 5.2.3: Illustration of method for DMS retention index

5.2.4 Triangle Test

This test was executed as per Watts et al., (94) using a basic sensory method. This method was executed to determine if consumers could differentiate between beers made from craft and pneumatic malt. Each participant was given three coded samples, palate cleansers and a ballot. Out of which two coded samples were the same beer and one

was odd. Each panelist was given different combinations in a sensory testing facility structured as per ASTM STP 913 and asked to examine the samples (98). The Taste Panel Ballot had necessary instructions in it and panelists were asked to select the odd sample.

5.2.5 Training the Panel

A total of 10 untrained panelists participated in DMS threshold take off evaluation in beer made from floor malt. Prior to testing, the panelists were trained to identify the overcooked cabbage or onion like flavour in beer. The sensory panel was trained according to the attribute rating approach from ASTM STP 758 method (95). A lightly hopped craft beer was used for training session. To acquaint the panelists with DMS flavour, samples of canned creamed corn in a plastic glass to sniff and taste were given to the panelists. Once they identified the flavour, they were asked to give a descriptor of their perception of DMS flavour and record it on the ballot provided. The panelists were further presented with a sample threshold test with the same beer spiked with 50.0, 85.5 or 100.0 µg/L of DMS along with one blank sample and palate cleansers. Each coded sample was rated on a scale of 0-8, and an open discussion was conducted regarding the same. All questions by panelists were answered to affirm their perception of DMS and to successfully carry out further trials. A majority of the panelists could identify DMS, it was concluded that nine panelists were fit for threshold testing.

5.2.6 DMS take-off threshold

This method proposed by Marin et al., (96) was applied to evaluate the maximum curvature point on an S-curve. The maximum curvature point would correlate to the least concentration of DMS (µg/L) in beer that a panelist was able to identify. With regard to trial threshold tests, the final range selected for actual tests was 30-150 µg of DMS in 1.0 L of beer. Each threshold test was divided into two days, in order to avoid sensory fatigue.

The DMS stock solution and samples were prepared freshly an hour prior each sensory session. Beer samples were spiked with food-grade DMS capsules according to the directions provided by Aroxa DMS Flavour Standard manual (Cara Technology, Leatherhead, Surrey, UK). These capsules consisted of 225.0 µg particulate DMS in them, but the chances of spillage of some particles while opening the capsule are very high. Also, DMS is known to be a very sticky compound, so once DMS was poured into the beer in a measuring flask, possibly some of the DMS might get stuck to the walls of the flask even after thorough mixing. Additionally, random minor measuring errors while pouring out the DMS spiked samples into growlers were expected and spillage of some DMS spiked samples was noted as well. Which indicates that the samples had relatively less DMS than expected (minor errors only). The samples were served according to ASTM E1871 method (97). The samples were given to the panelists in random order with coded random three-digit numbers. First day included samples containing 30.0, 60.0, 80.0 and 90.0 µg/L DMS, one blank and palate cleansers along with the ballot. The second day of trial included samples containing 100.0, 120.0 and 150.0 µg/L DMS, one blank and palate cleansers. Illustration of ballot used for threshold testing is presented in Figure 5.2.6 below.

Threshold Trial- 5
9-point scale for DMS flavour intensity in beer

Date: July 11th, 2019

Name:

You have received one control and 3 beer samples; the 3 samples contain dimethyl sulphide (DMS). The samples are in random order, for each sample, please indicate the size of difference from control by checking one of the boxes below.

On the given scale, 0 = no difference and 8 = extremely different.

	Difference Scale								
Sample code	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

Figure 5.2.6: DMS threshold evaluation ballot

5.2.7 Colour

The colour of the pilot brews and the lab fermented wort samples was measured according to ASBC method Beer-10A (99). Beer and wort samples were filtered with filter paper (9.0-cm, VWR-5, PA) to degas and remove yeast and other particles. The filtrate was transferred to disposable plastic cuvettes with sterile transfer pipettes. Absorbance readings were taken at 430.0 nm in triplicate and colour was reported in degree SRM.

5.2.8 Plato Determination

The specific gravity of wort was determined with a digital density meter (DMA 35N, Anton Paar GMbH, Graz, AUT) at 21.0ºC by following the manufacturer's manual and the standard ASBC method Beer-2 (100).

5.2.9 Turbidity

From each miniature fermentation test tube 3.5 mL of wort was transferred to a disposable four-sided cuvette and the absorbance was measured at 600.0 nm at an ambient temperature of 21.0ºC as mentioned in ASBC assay Yeast-14 (89).

6 Results

6.1 Germination Quality

Following are the values obtained for all the germination experiments conducted in triplicate. Refer to Table 6.1 and Figure 6.1. In table 6.1, the replicates indicate that each experiment was triplicated. The average and standard deviation displayed below represent the three replicates for each GC, GE and WS, respectively.

Table 6.1: Germination quality of two-row CDC Copeland barley (n = 3)

GC (%)	Average (%)	Standard Deviation	GE (%)	Average (%)	Standard Deviation	WS (%)	Average (%)	Standard Deviation
99.0	96.33	1.89	100.0	100.0	0.00	3.0	5.67	4.50
95.0			100.0			12.0		
95.0			100.0			2.0		

6.1.1 Germinative Capacity

Germinative capacity for two-row CDC Copeland variety was assessed according to the hydrogen peroxide method by ASBC, performed in triplicate (91). The germination capacity observed for our two-row CDC Copeland variety was 99.0, 95.0 and 95.0, $\pm$ 1.90% respectively (Table 6.1 and Figure 6.1). ASBC reported a combined-laboratory error ranging from 1.8 to 2.8% for germinative capacity – hydrogen peroxide method. A high germination capacity around 98% is a desired attribute of seeds for production of good quality malts. This test indicates if the crop has overcome the state of dormancy in order to be malted. A low germinative energy suggests that viability of grain may have been reduced by insect attack, exposure to excessive temperature during drying,

physical damage and inappropriate storage conditions (12). The present results suggest that this particular batch of barley displays sufficient germinative capacity and is suitable for malting.

6.1.2 Germinative Energy

Germinative energy for two-row CDC Copeland variety was evaluated according to ASBC simultaneous determination method and was performed in triplicate (91). Germinative energy exhibits the vigor with which barley germinates. Germinative energy measured for our two-row CDC Copeland variety in triplicate was 100.0% (Table 6.1 and Figure 6.1).

Kegge et al., (106) reported a germinative energy of 97% (in duplicate) for two-row hulled CDC Copeland barley. While MacLeod et al., (107) evaluated germinative capacities for two-row CDC Copeland barley germinated in Saskatchewan as 99 and 96%, Alberta as 99, 90 and 99% and Prairies as 96 and 98%, respectively. Additionally, values of GE in excess of 95% are desired (110). Hence, it can be established that this individual batch of two-row CDC Copeland possesses high germinative ability at a temperature range of 18.0-20.0^{o}C.

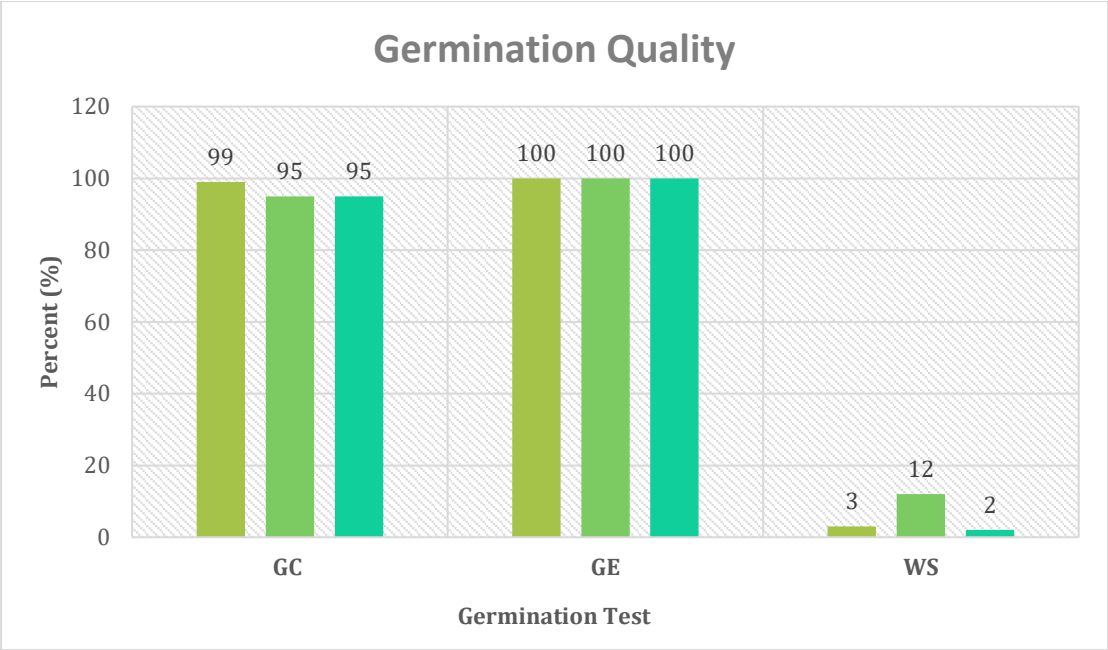
6.1.3 Water Sensitivity

Water sensitivity for two-row CDC Copeland variety was measured according to ASBC simultaneous determination method and was performed in triplicate (91). The term water sensitivity was coined by Matthews and Collins (108) and refers to abatement in germinability of seeds during excessive water conditions. Seed sensitivity is majorly

affected by water deficiency and abundance. Water sensitivity recorded by Hosnedl et al., (109) ranged from 40.0-91.7% over the period of seven years in different barley seed samples. However, water sensitivity for two-row CDC Copeland barley evaluated through the data provided by MacLeod et al., (107) germinated in Saskatchewan was 10%, Alberta was 11% and Prairies was 9%, respectively. While water sensitivity for two-row CDC Copeland variety in the present study was established as 3.0, 12.0 and 2.0 $\pm$ 4.5%, respectively (Table 6.1 and Figure 6.1). Water sensitivity percentage of 25 or more is indicative of severe water stress (112).

Water sensitivity may vary based on barley genotype and presence of hull. It was observed that the "glue" between the hull and caryopsis limits the exchange of gases and water which ultimately reduces the germinability of the barley grains (137). The two-row Copeland CDC used for the present experimentation was also hulled, however, considering the water sensitivity values obtained, it can be concluded that the grains were not extensively prone to water sensitivity.

It should be noted that this barley was stored in a dry polyethylene storage case, at a temperature of 18.0-20.0^{o}C. The purpose of these post-harvest germination tests was to determine if barley had overcome dormancy adequately and proven to be suitable for malting. Careful review of the data obtained (Table 6.1 and Figure 6.1), indicated that two-row CDC Copeland, hulled barley was suitable for malting. No alarming variation among the replicates of each germination test was observed. Each bar in the figure represents an individual value for germinative capacity (GC), germinative energy (GE) and water sensitivity (WS), respectively.



(n = 3) GC = 96.33 ± 1.89%, GE = 100.0 ± 0.00% and WS = 5.67 ± 4.50%

Figure 6.1: Germination quality of two-row CDC Copeland barley

In order to supply first grade malt, malting barley cultivars have been bred for high levels of malt extract, diastatic power, α-amylase and free amino nitrogen according to guidelines set by American Malting Barley Association (110). To achieve this malt, adequate germination of barley is must. Results of germination vary often depending on germination temperature, water and oxygen availability, barley genotype, seasonal variations and storage conditions (12, 111). To determine germinative abilities of barley, performing germination experiments is requisite. Brower et al., (112) reported GC, GE and WS (%) of two row CDC Copeland Barley as 94, 93 and 8 %, respectively. Corresponding to which, it can be reported that two-row CDC Copeland barley selected for our research was overall a suitable candidate for malting.

6.2 Calibration of fibre for detection of DMS

Six calibration standards were prepared with analytical grade DMS (≥ 98% GC, Sigma Aldrich, Oakville, ON) concentrations of 0.0, 2.0, 4.0, 6.0 and 8.0, 12.0 and 16.0 µg/L with 8.0 mL of distilled water in glass vials. Solutions were mixed immediately, and vials were sealed tightly. Each vial was placed on a digital dry bath at 40.0ºC for 30.0 min, with a 1.0 cm DVB-CAR-PDMS fibre (Supelco, Sigma Aldrich, Oakville, ON) inserted into the headspace. A calibration curve for DMS was prepared by the data obtained from the chromatogram as suggested by ASBC method Malt 14 (92). Calibration was performed periodically and resulted in $r^2 = 0.96$ (Figure 6.2).

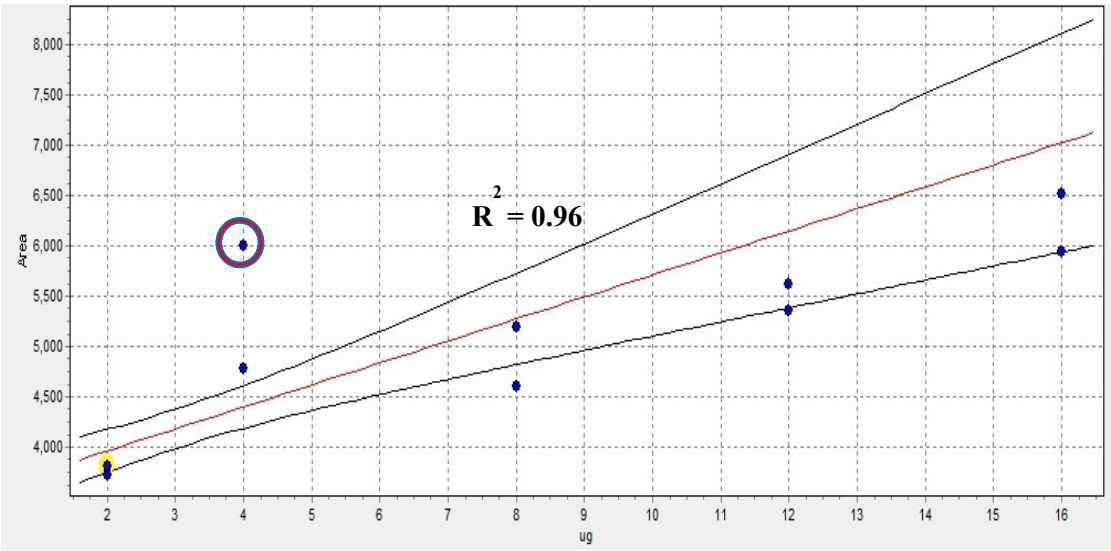


Figure 6.2: Calibration curve for DMS

With reference to the calibration curve above, the replicate area point encircled for standard concentration 4.0 µg/L is considerably out of range, which indicates towards a discrepancy of this particular standard. It was deleted from the calibration but is reported here to illustrate the difficulty in this calibration. Even though the calibration

was run periodically, there could be several reasons for this error. First, DMS is a very viscous compound that tends to stick to the surface, so chances of DMS sticking to the vial tops, vial screw caps and pipette tips are high. There is a possibility of pipetting error or a random error while preparing the standards. DMS is a highly volatile sulphur compound which is problematic with regard to detection because of its presence in low concentrations and more so because of its low chemical stability (145). This issue with calibration runs was addressed by Lopez et al., (144) who conducted single component standard addition experiments and observed that the decrease in areas of standards was less important (20%) but linearity issues persisted. In their standard addition calibration plots, they recorded a trend which implied towards saturation. Furthermore, this saturation was not applicable for the detector as the effect surfaced even when the signal was at 20% of the full scale, which led them to conclude that generating an acceptable linear response required restriction of concentration range.

Hence, it could be speculated that the difficult nature of DMS delivery when combined with random lab errors and saturation could result in erratic outliers.

6.3 Retention Index of DMS

Kovats retention indices are retention times normalized to adjacently eluting n-alkanes. The objective of calculation of RI is for the absolute identification of a particular analyte in the chromatogram. A 150.0 uL microinjection needle was used to inject analytical grade hexane and decane (≥ 98% GC, Sigma Aldrich, Oakville, ON) into filter papers, which was then placed in a 6 L Erlenmeyer flask. The DVB-CAR-PDMS fibre was then inserted in the headspace of flask for 30.0 min and later injected in the GC. The run was

continued for 12.0 min. The retention times and indices of hexane, DMS and decane were predetermined through the NIST library. However, retention times and index may slightly vary depending on their extraction method, GC equipment and column which necessitates determination of the Kovats Retention Index (Figure 6.3).

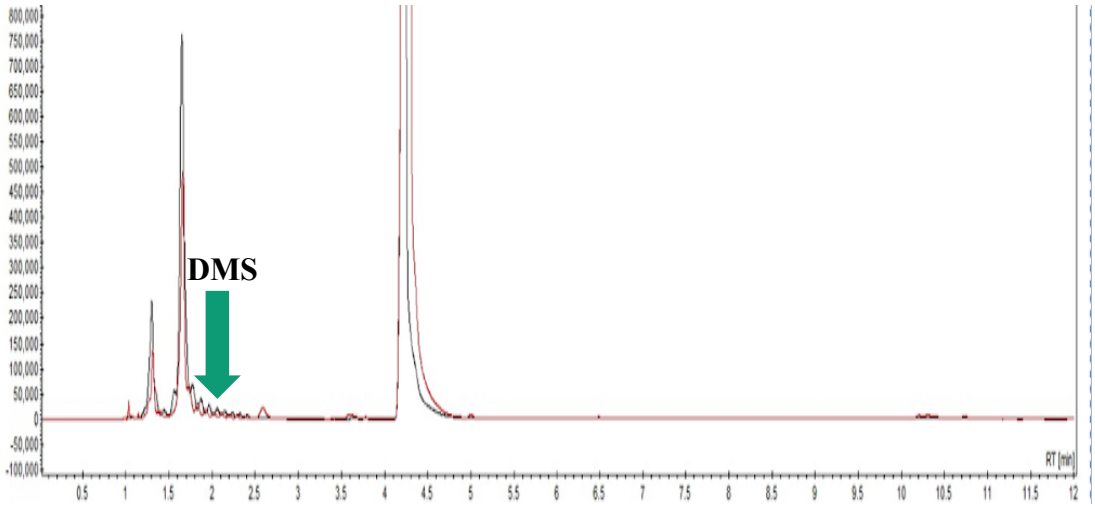


Figure 6.3: Retention of DMS at 2.07 min

It was observed that hexane has a retention time of 1.5 min while decane had a retention time of 4.2 min. Retention indices of hexane and decane were 600.0 and 1000.0, respectively (157, 158), while DMS eluted at 2.07 min with a retention index of 752.0.

6.4 DMSP in Malt

6.4.1 DMSP in Green Malt

6.4.1.1 DMSP in Green Malt Germinated at 10°C

Germination of green malt at 10.0°C took five days to complete. Triplicate batches of green malt were produced. For each batch, two samples were analyzed, hence a total of

six samples of green malt germinated at 10.0ºC were tested for presence of DMSP by HS-SPME-GC-FID. (Table 6.4.1.1)

Table 6.4.1.1: DMS and DMSP values in green malt germinated at 10.0ºC (n = 3)

Batch	Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	DMSP (µg/g)	Average (µg/g)	Standard Deviation
1	6.54	10.6	5.14	n/d	6.54	10.6	5.14
	8.66			n/d	8.66		
2	6.11			n/d	6.11		
	20.13			n/d	20.13		
3	11.56			n/d	11.56		
	n/d*			n/d	n/d		

LOD = 0.81 ng/g or 0.00081µg/g, n/d indicates <0.00081 µg/g.

*The n/d obtained in duplicate total DMS sample taken from the third batch, could be disregarded because no other curve was seen in the chromatogram. This possibly could be just an erratic run or a result of seed to seed variation. No free DMS was detected in green malt, thus, amounts of DMSP obtained were the same as total DMS.

6.4.1.2 DMSP in Green Malt Germinated at 17.5ºC

Germination of green malt at 17.5ºC took four days to complete. Triplicate batches of green malt were produced. For each batch, two samples were analyzed, hence a total of six samples of green malt germinated at 17.5ºC were tested for presence of DMSP by HS-SPME-GC-FID. (Table 6.4.1.2)

Table 6.4.1.2: DMS and DMSP values in green malt germinated at 17.5ºC (n = 3)

Batch	Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	DMSP (µg/g)	Average (µg/g)	Standard Deviation
1	21.12	11.16	5.70	n/d	21.12	11.16	5.70
	15.69			n/d	15.69		
2	11.3			n/d	11.3		
	7.89			n/d	7.89		
3	5.68			n/d	5.68		
	5.26			n/d	5.26		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g.

No free DMS was detected in green malt, thus, amounts of DMSP obtained were the same as total DMS.

6.4.1.3 DMSP in Green Malt Germinated at 25ºC

Germination of green malt at 25.0ºC took four days to complete. Triplicate batches of green malt were produced. For each batch, two samples were analyzed, hence a total of six samples of green malt germinated at 25.0ºC were tested for presence of DMSP by HS-SPME-GC-FID. (Table 6.4.1.3)

Table 6.4.1.3: DMS and DMSP values in green malt germinated at 25.0ºC (n = 3)

Batch	Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	DMSP (µg/g)	Average (µg/g)	Standard Deviation
1	24.95*	6.92	9.98	n/d	24.95	6.92	9.98
	n/d*			n/d	n/d		
2	n/d*			n/d	n/d		
	0.36*			n/d	0.36		
3	n/d*			n/d	n/d		
	16.21*			n/d	16.21		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g.

*Extreme variation was observed in total DMS samples, which indicates uncontrolled random errors during the experimentation. It should also be noted that these samples were processed essentially in the same manner as other samples. Practically, there could be saturation of fibre and/or detector which resulted in such discrepant values. After all sets of samples were tested, a new set of calibration was run, which showed that the detectability of fibres was decreasing over time possibly because of viscous nature of DMS which resulted in saturation of fibres. Additionally, sulphur compounds are extremely sensitive to oxidants and thus are prone to oxidation into different compounds (145). Exposure to minute quantities of light or metal can lead to oxidation of sulphur compounds and result in significant losses of analytes during the different steps of the analysis. Formation of dimethyl sulfoxide from DMS in the course of the analysis or during the chromatographic injection, is well documented in the literature (147, 148). No free DMS was detected in green malt, thus, amounts of DMSP obtained were the same as total DMS.

Although there was a great deal of variability within and between batches at each temperature, the greatest levels of total DMS or DMSP were found in green malt germinated at 25.0°C i.e., 25.0 ± 10.0 µg/g, followed by 17.5°C with 21.1 ± 5.70 µg/g, and 10.0°C yielding 20.0 ± 5.14 µg/g DMSP, respectively. This corroborates to the statement made by Kavanaugh et al., (4) that germination temperatures above 20.0°C induce formation of DMSP in malt.

Previous research showed that green malts germinated at 20.0°C contained 9.3 µg/g DMSP (141). Relatively, the malts in the present study produced at all germination temperatures produced greater amounts of DMSP. Scherb et al., (138) measured SMM through steeping, germination and malting and after four days of germination observed that, SMM levels increased to 24.0 mg/kg. While wort produced from green malts consisted DMSP levels of 822.0 µg/L and after 70.0 hours of fermentation, 38.0 µg/L of DMS was obtained in beer (5).

6.4.2 DMSP in Kilned Malt

6.4.2.1 DMSP in Kilned Malt Germinated at 10°C

Green malt germinated at 10.0°C was kilned at 63.0°C for 24.0 hours to produce a pale ale malt. Triplicate batches of kilned malt were produced. For each batch, two samples were analyzed, hence a total of six samples of kilned malt germinated at 10.0°C were tested for the presence of DMSP by HS-SPME-GC-FID (Table 6.4.2.1).

Table 6.4.2.1: DMS and DMSP values in kilned malt germinated at 10.0ºC (n = 3)

Batch	Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	Average (µg/g)	Standard Deviation	DMSP (µg/g)	Average (µg/g)	Standard Deviation
1	n/d	11.70	10.50	n/d	3.55	7.31	n/d	8.14	4.60
	5.21			n/d			5.21		
2	8.82			1.43			7.39		
	33.38			19.86			13.52		
3	12.17			n/d			12.17		
	10.54			n/d			10.54		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g.

6.4.2.2 DMSP in Kilned Malt Germinated at 17.5ºC

Green malt germinated at 17.5ºC was kilned at 63.0ºC for 24.0 hours to produce a pale ale malt. Triplicate batches of kilned malt were produced. For each batch, two samples were analyzed, hence a total of six samples of kilned malt germinated at 17.5ºC were tested for the presence of DMSP by HS-SPME-GC-FID (Table 6.4.2.2).

Table 6.4.2.2: DMS and DMSP values in kilned malt germinated at 17.5ºC (n = 3)

Batch	Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	Average (µg/g)	Standard Deviation	DMSP (µg/g)	Average (µg/g)	Standard Deviation
1	22.23	17.55	5.34	10.02	8.13	6.64	12.21	9.42	9.83
	15.04			3.16			11.88		
2	14.27			n/d*			14.27		
	9.33			21.04*			11.71*		
3	18.98			8.28			10.70		
	25.44			6.29			19.15		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g. *For one replicate of batch two, the amount of free DMS obtained was more than the total DMS. In theory, DMSP is equal to the difference of free DMS from total DMS, which resulted in the DMSP value of -11.71.

The relatively low amount of total DMS in this case could potentially be because of saturation of the fibre in between these runs. The fibre was conditioned between total and free DMS samples but not within each total or free DMS type run. For free DMS, it was noticed that for first replicate of batch two, no free DMS was detected and for the second replicate, the free DMS levels were unusually high with respect to other samples, it was suspected that DMS from the first replicate of batch 2 was carried over from the fibre to the second replicate run, which resulted in the values above. These replicates were not meant to be tested in their actual order as all the samples were coded and randomized to avoid bias. Which could also lead to two similar samples being tested subsequently.

6.4.2.3 DMSP in Kilned Malt Germinated at 25°C

Green malt germinated at 25.0°C were kilned at 63.0°C for 24.0 hours to produce a pale ale malt. Triplicate batches of kilned malt were produced. For each batch, two samples were analyzed, hence a total of six samples of kilned malt germinated at 25.0°C were tested for presence of DMSP by HS-SPME-GC-FID (Table 6.4.2.3).

Table 6.4.2.3: DMS and DMSP values in kilned malt germinated at 25.0°C (n = 3)

Batch	Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	Average (µg/g)	Standard Deviation	DMSP (µg/g)	Average (µg/g)	Standard Deviation
1	20.64	12.61	5.90	12.74	4.51	4.12	7.9	8.11	6.00
	9.21			3.34			5.87		
2	8.93			2.36			6.57		
	21.08			1.01			20.07		
3	6.74			6.51			0.23		
	9.08			1.08			8.00		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g.

The greatest levels of DMSP were found in kilned malt germinated at 17.5°C i.e., 14.3 ± 9.83 µg/g, followed by 10.0°C with 13.5 ± 4.6 µg/g, and 25.0°C yielding 8.0 ± 6.0 µg/g DMSP, respectively. Scherb et al., (138) stated that as a consequence of kilning, levels of SMM in green malts reduced by 90% from 24.0 mg/kg SMM. Contrary to this, a reduction of 32.0, 68.0 and 68.0% was observed in our DMSP levels for malts germinated at 25.0, 17.5 and 10.0°C, respectively in all our kilned malt samples.

White and Wainwright (5) displayed that malts kilned at 65.0ºC for 24.0 hours gave 8.3 µg/g of DMSP, however in beer DMS levels reached up to 60.0 µg/L because during fermentation DMS was being formed at the rate of 35.0 µg/L from day one of fermentation. It also suggests, that this kilning regime did not sufficiently deplete all the DMSP produced. Because a similar kilning regime was applied to our malt samples, it could be assumed that the DMSP may not have been degraded consummately.

6.4.3 Comparison between Total DMS in Green and Kilned Malt

A two-way ANOVA was carried out on green and kilned malt samples obtained from each germination temperature (Table 6.4.3 and Figure 6.4.3).

Table 6.4.3: Comparison of total DMS in green and kilned malt

Two-way Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
Green v/s Kilned	134.41	1	134.41	1.96	0.17
Temperature	242.42	2	121.21	1.77	0.19
Process*temp.	29.12	2	14.56	0.21	0.81
Error	2052.92	30	68.43		

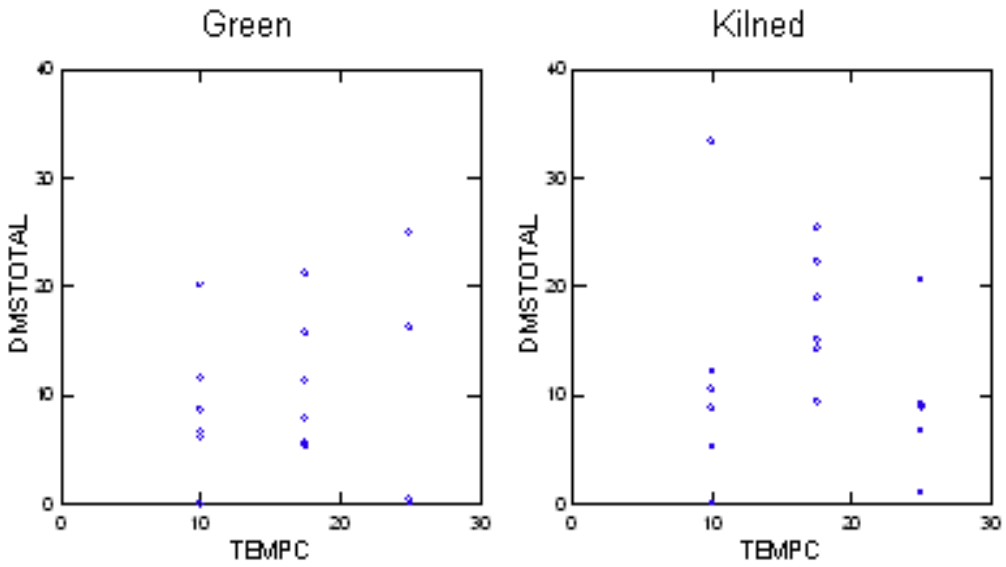


Figure 6.4.3: Scatter plot of total DMS in green and kilned malt (n = 9)

From the Table and Figure 6.4.3 for total DMS present in all green and kilned malt samples, it can be concluded that:

- Levels of total DMS obtained from green and kilned malts were not significantly different (p-value > 0.05), (i.e., kilning did not reduce total DMS concentration significantly).
- Total DMS levels within each germination temperature were not significantly different (p-value > 0.05), (i.e., germination of malt at 10.0, 17.5 and 25.0^{o}C did not affect the concentration of total DMS present in all the malt samples).
- Total DMS levels measured between green malt germinated at three temperatures and kilned malt obtained from those green malts, were not significantly different (p-value > 0.05).

6.4.4 Comparison between Free DMS in Green and Kilned Malt

A two-way ANOVA was carried out on green and kilned malt samples obtained from each germination temperature (Table 6.4.4 & Figure 6.4.4).

Table 6.4.4: Comparison of free DMS in green and kilned malt

Two-way Analysis of Variance					
Source	**Type III SS**	**df**	**Mean Squares**	**F-Ratio**	**p-Value**
Green v/s Kilned	381.50	1	381.50	13.00	0.00113
Temperature	39.60	2	19.80	0.67	0.52
Process*temp.	39.60	2	19.80	0.67	0.52
Error	882.42	30	29.41		

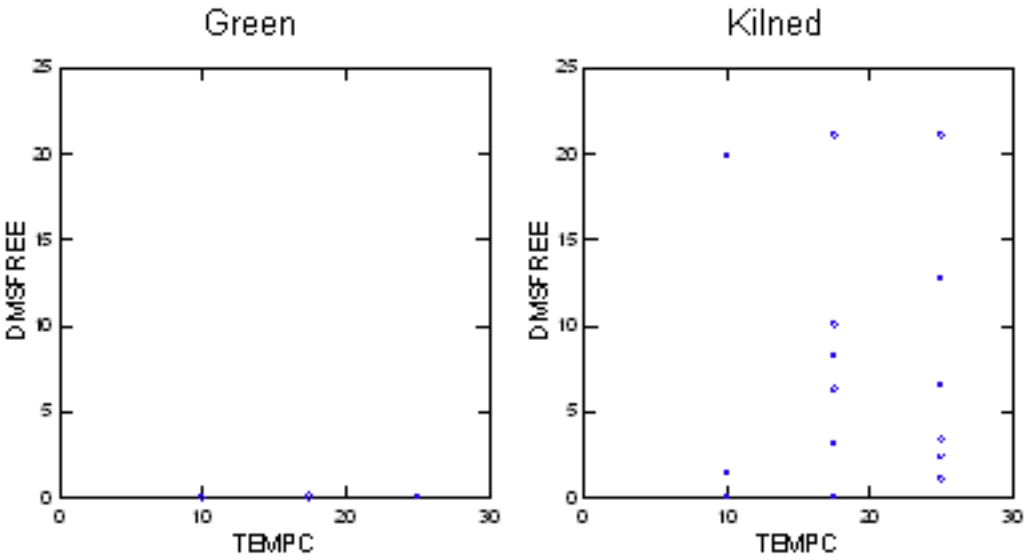


Figure 6.4.4: Scatter plot of free DMS in green and kilned malt (n = 9)

From the Table 6.4.4 and Figure 6.4.4 above for free DMS present in all green and kilned malt samples, it can be comprehended that:

- Levels of free DMS obtained from green and kilned malts were significantly different (p-value < 0.05). (i.e., Kilning resulted in significant evolution of free DMS in kilned samples as opposed to green malt samples).
- Free DMS levels within each germination temperature were not significantly different (p-value > 0.05). (i.e., Germination of malt at 10.0, 17.5 and 25.0ºC did not affect the concentration of Free DMS present in all malt samples).
- Free DMS levels measured between green malt germinated at three temperatures and kilned malt obtained from those green malts, were not significantly different (p-value > 0.05).

6.4.5 Comparison of DMSP in Green and Kilned Malt

A two-way ANOVA was carried out on green and kilned malt samples obtained from each germination temperature (Table 6.4.5).

Table 6.4.5: Comparison of DMSP in green and kilned malt

Analysis of Variance

Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
Green v/s Kilned	63.02	1	63.02	0.81	0.34
Temperature	237.30	2	118.64	1.53	0.23
Process*temp.	38.40	2	19.19	0.25	0.78
Error	2320.50	30	77.40		

From the Table 6.4.5 above for DMSP present in all green and kilned malt samples, it can be concluded that:

- Levels of DMSP obtained from green and kilned malts were not significantly different (p-value > 0.05). (i.e., Kilning did not result in significant reduction of DMSP).
- DMSP levels within each germination were not significantly different (p-value > 0.05). (i.e., Germination of malt at 10.0, 17.5 and 25.0^{o}C did not affect the concentration of DMSP present in all malt samples).
- DMSP levels measured between green malt germinated at 3 temperatures and kilned malt obtained from those green malts, were not significantly different (p-value > 0.05).

6.5 DMSP in Fermented Wort

Kilned malt germinated at 10.0, 17.5 and 25.0^{o}C was mashed and mini-fermented in laboratory as per ASBC Yeast-14 assay to obtain fermented wort. For each germination temperature, triplicate batches of malt were mashed and ultimately six tubes of wort were collected. Overall 18 wort samples (six for each germination temperature) were prepared, filtered and stored at -78.0^{o}C.

All these wort samples were assayed by the same HS-SPME-GC-FID method employed for the malt samples in order to maintain uniformity. However, no total or free DMS was detected in any of the vials. Hence, DMSP levels could not be quantified in fermented wort samples (Figure 6.5).

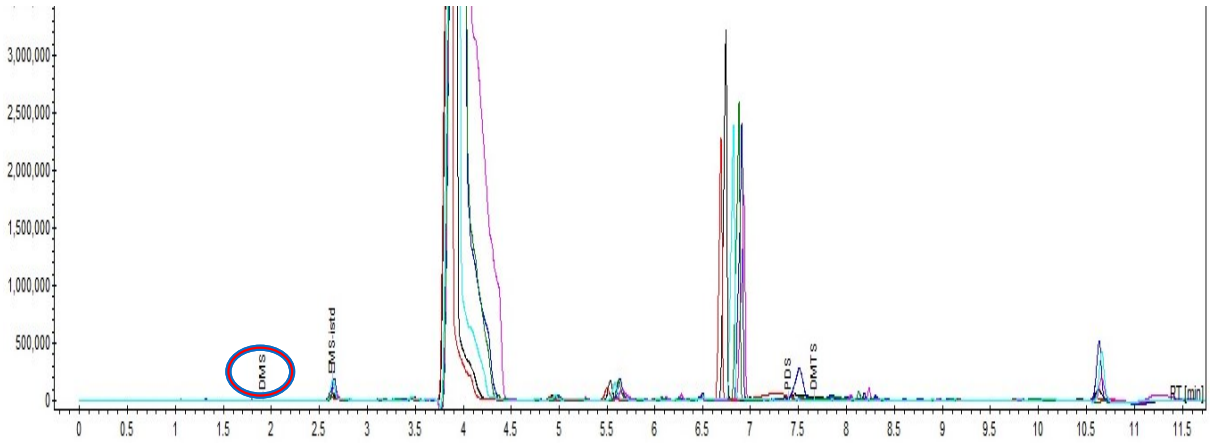


Figure 6.5: DMS in fermented wort

The peak at 2.07 min illustrates that no total or free DMS was detected, hence, no DMSP was detected in lab fermented wort samples. It should be noted that the method was calibrated ($r^2 = 0.96$) periodically for detection of miniscule levels of DMS ranging from 2-16.0 µg/L. A non-significant drop (p-value > 0.05) was observed in levels of DMSP from green to kilned malts (Tables 6.4.1.1 to 6.4.2.3).

In the mini-fermentation assay, malts were first mashed, then filtered to obtain wort, which was further autoclaved and pitched with yeast. Possibly, the autoclaving of wort at 121.0ºC under high pressure of 1.02 atm degraded all the DMSP left over from kilning. Considering these malts did not have high levels of DMSP to begin with, not much would be left after autoclaving to be detected by the GC.

It should also be noted that the mini fermentation tubes are only sealed with sponge bungs after pitching the yeast. Potentially, some of the DMS would also evolve and escape the tubes during fermentation which may explain why no DMSP levels were

reported in wort samples. It should also be noted that for storage, wort samples were filtered before freezing, and possibly, filtration got rid of all the yeast cells which corroborates to the absence of DMSP in wort as filtration eliminates the possibility of evolution of DMS from DMSO.

During preparation of wort, MetSO is also produced. DMSO reductase is relatively more affinitive towards MetSO than DMSO. As a result of which, DMSO may not be reduced, hence no DMS will be produced in the wort. DMSO reductase activity is also reduced by presence of nitrogen (139).

In brewery-scale fermentations, a 64 to 72.0% reduction in DMS with a mean of 69.0% in nine fermentations was observed (140).

A research showed that malt kilned at 65.0^{o}C for 24.0 hours yielded DMSP levels of 271.0 µg/L which lead to evolution of 61.0 µg/L after 70.0 hours of fermentation (5). However, in our case, even though the kilning regime was similar, similar levels of DMS were not obtained.

6.6 DMSP in Floor Malt

Floor malt was germinated in a non-temperature-controlled environment where the average temperature recorded was 19.0^{o}C while the maximum temperature reported was 26.0^{o}C and kilned at maximum temperature of 80.0^{o}C to produce a pale ale malt with moisture below 6.0%. Six samples of this floor malt were investigated for presence of DMSP by HS-SPME-GC-FID (Table 6.6).

Table 6.6: DMS and DMSP values in floor malt (n = 6)

Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	DMSP (µg/g)	Average (µg/g)	Standard Deviation
10.75	6.94	2.80	n/d	10.75	6.94	2.80
5.98			n/d	5.98		
3.31			n/d	3.31		
3.96			n/d	3.96		
7.8			n/d	7.8		
9.85			n/d	9.85		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g.

No free DMS was detected, thus, amounts of DMSP obtained were the same as total DMS.

6.7 DMSP in Pneumatic Malt

Pneumatic malt was germinated at 14.0ºC for 96.0 hours in a temperature-controlled environment. This malt was kilned at a maximum temperature of 80.0ºC to produce a pale ale malt with moisture below 6%. Six samples of this pneumatic malt were analyzed for presence of DMSP by HS-SPME-GC-FID (Table 6.7).

Table 6.7: DMS and DMSP values in pneumatic malt (n = 6)

Total DMS (µg/g)	Average (µg/g)	Standard Deviation	Free DMS (µg/g)	Average (µg/g)	Standard Deviation	DMSP (µg/g)	Average (µg/g)	Standard Deviation
28.58	32.20	8.20	12.88	10.71	3.15	15.7	21.50	10.30
30.3			5.79			24.51		
23.78			14.88			8.90		
26.03			11.27			14.76		
48.45			7.39			41.06		
35.90			12.02			23.88		

LOD = 0.81 ng/g or 0.00081 µg/g, n/d indicates <0.00081 µg/g.

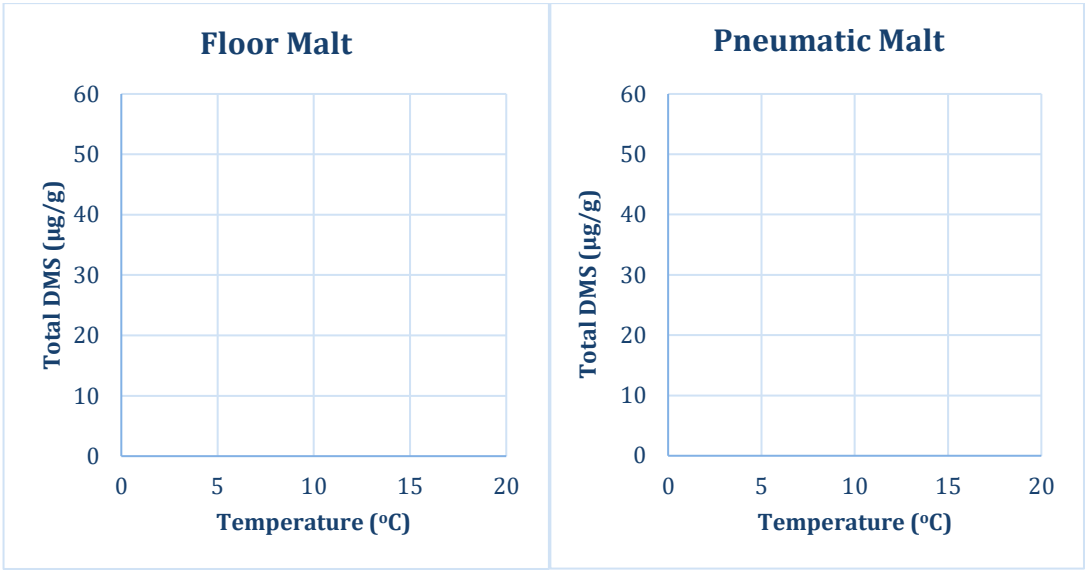
6.8 Comparison of DMSP in Floor and Pneumatic Malt

The impact of the two malting processes on generation of DMSP was evaluated by measuring DMSP through HS-SPME-GC-FID in both commercially available floor and pneumatic malt (Table 6.8, Figure 6.8.1 and 6.8.2). After measuring the amounts of DMSP in each, the highest amounts of DMSP in floor and pneumatic malt were 10.75 $\pm$ 2.8 and 41.0 $\pm$ 10.3 µg/g, respectively, i.e., DMSP in pneumatic malt was almost four times the amount of DMSP detected in floor malt.

Table 6.8: Comparison of DMSP in floor and pneumatic malt

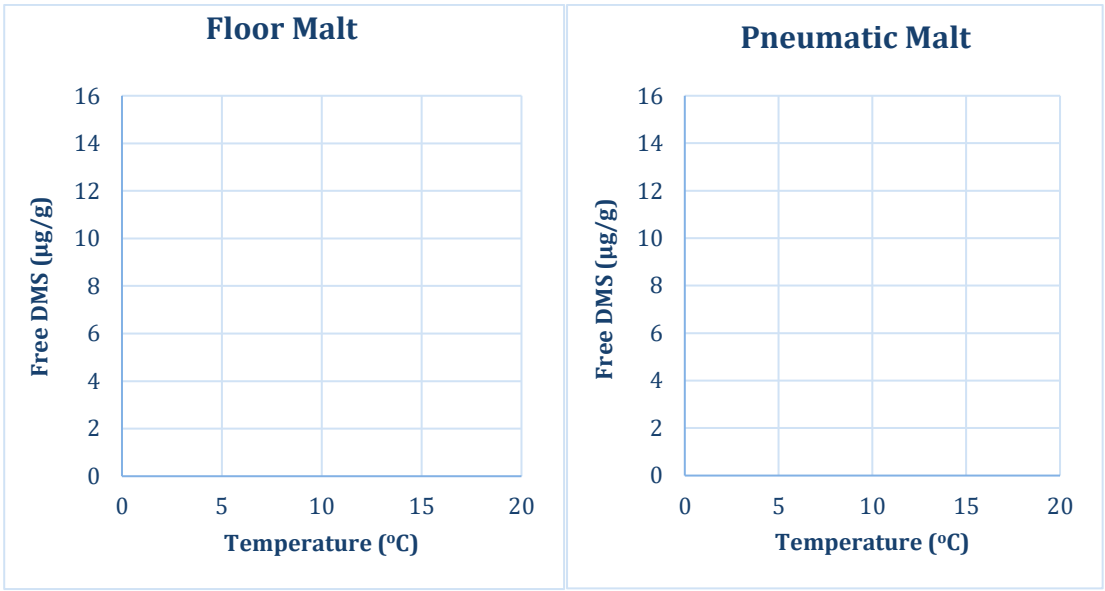
Analysis of Variance					
Source	**Degrees of Freedom (DF)**	**Sum of Squares (SS)**	**Mean Square (MS)**	**F-stat**	**p-value**
Between Groups	1	626.40	626.40	9.17	0.013
Within Groups	10	683.20	68.30		
Total:	11	1309.60			

Furthermore, one-way analysis of variance exhibited a significant difference between Total and Free DMS and DMSP obtained by both malts with a p-value of 0.013 (p-value < 0.05). This reveals that the malting treatments have a significant impact on DMSP levels generated in the malts. However, it was expected the more DMSP would be produced in floor malt than pneumatic malt suggesting that in this particular case germination temperature may not be the primary cause of the origination of DMSP.



N.B. (n = 6) Average total DMS: Floor malt = 6.94 ± 2.8 µg/g and Pneumatic malt = 21.5 ± 10.3µg/g.

Figure 6.8.1: Scatter plot of total DMS in floor and pneumatic malt



N.B. (n = 6) Average free DMS: Floor malt = n/d indicates <0.00081µg/g, Pneumatic malt = 10.71 ± 3.15µg/g.

Figure 6.8.2: Scatter plot of free DMS in floor and pneumatic malt

6.9 Sensory Analysis

6.9.1 Triangle Test

Pneumatic and floor malt APA with a target alcohol by volume of 5.6% and 12-15 IBU were prepared in duplicate on two separate days. In order to discern the effect of each malt on beer flavour, a group of untrained panelists was selected. This was done to understand consumer's perspective of beer flavour.

Replicate 1

A total of 14 untrained panelists participated in this test, out of which eight people were able to identify the odd sample in a triangle test. A one-tailed binomial test revealed a p-value of 0.058 which is greater than 0.050. Hence, it was established that the two beers were not significantly different from a regular beer consumer's perspective.

Replicate 2

Six untrained panelists participated in this test, out of which only three people were able to identify the odd sample in a triangle test. A one tailed-binomial test generated a p-value of 0.320 which is greater than 0.050. Accordingly, it was recorded that the duplicate batches of the two APA were not significantly different from a regular beer consumer's perspective.

6.9.2 Training the Sensory Panel

To narrow down the range of DMS (µg/L) actively detected by the panelists, we gave them beer samples containing 5-150.0 µg/L of DMS over a period of four days. These trial threshold tests were conducted as per the method of Marin et al., (96) to determine the panelists dose response. The present data obtained was especially noisy, as the panel was untrained. However, we observed that it required the addition of 12 to 27.0 µg/L of DMS to the beer to reach the threshold for detection.

Hence, we trained our 10 panelists to identify flavour of DMS as per the ASTM STP 758; attribute rating method. A lightly hopped, herbal and citrusy craft beer was spiked with 50.0 µg/L, 85.5 µg/L and 100.0 µg/L DMS. Out of ten, nine panelists ranked all the samples on a scale of 0-8 correctly based on their difference from blank. Hence, these nine panelists were deemed suitable for a series of DMS threshold measurement in floor malt APA. With regard to trial threshold tests, the final range selected for actual tests was 30.0-150.0 µg of DMS in 1.0L of beer.

6.9.3 DMS take-off threshold

DMS Take-Off Threshold experiments were replicated four times as recommended, however, the data obtained for each session was drastically different for each participant and could not fit in logistic model presented by Marin et al., 1991 (96).

Schutte and Roy (101) stated that sulphur containing flavour compounds can predominate overall flavour perception of foods by conferring overpowering and peculiar odour at very low threshold values.

Threshold values of DMS in beer change in accordance with the beer's chemical composition and its endogenous DMS concentration (102). Several researchers have employed a variety of methods to evaluate DMS sensory threshold in beers, and reported a range of, 9-60 µg/L from a series of difference rating test (103). A low threshold of 25-30 µg/L was also noted (102) and 68-69 µg/L DMS in Canadian beers (105). Meilgaard (58, 6) demonstrated a range of DMS thresholds of 0.9-57 µg/L and 25-50 µg/L, respectively.

Nevertheless, inability of the participants to detect DMS in floor malt beer maybe due to the low levels of DMSP obtained in floor malt. These results do not support the hypothesis that beer made from floor malt may have high DMS levels which would result in beer with high DMS flavour threshold. Possible explanations for the observed data are:

- *Endogenous DMS concentration of blank (un-spiked beer)*- the panelists did not assign higher ranks to samples containing very high levels of DMS, indicating that they did not find a noticeable difference between those samples and the blank. This ultimately implies that the blank may have relative DMS levels similar to those samples.
- *Overall sensory fatigue*- a general trend noticed was that panelists gave high ratings to first sample they tasted, majority of times. After which they were not able to determine the difference quite efficiently. Possibly because DMS is a very intense flavour, and even with palate cleansers it saturates the panelist's taste and olfactory receptors.

- *Perceived intensity of DMS in craft beer*- craft beers tend to be on the more flavorful side of the spectrum, where masking off-flavours is relatively easier. Even though we used "optimal assessment technique" for evaluating DMS take-off threshold, its perceived intensity in craft beer would be practically much less than generic ales/lagers produced on a macro-scale.

Meilgaard et al., (103) found that frequency distribution for individual assessors was skewed towards the high side. For externally added DMS to beer consisting 3.5% alcohol, 85-90% of panelists had threshold in the range of 1 to 10 and were categorized as "normal" (N.B., threshold not mentioned by Meilgaard et al., 103) group. While rest of the panelists formed a "low sensitivity" group that had thresholds higher by 10 to 20 times of the lower threshold range. No participant was completely DMS blind. However, after repeating the experiment four times, they concluded that for any given substance, a small group of very insensitive individuals exists. Another factor that may affect the threshold evaluation is "Rosenthal effect", where panelists supply proof of non-existing effects to please the panel leader.

In this study, out of nine panelists only two panelists seemed to display a bearably "normal" threshold. While the other seven panelists could be classified as "low sensitivity" group, as frequency threshold for each participant in this group was inclined towards the higher range, as suggested by Meilgaard et al., (103) mentioned above. To obtain a reliable estimate of threshold, the experiment design should be feasible as well as provide 95% confidence limits (103). Hence, it can be concluded that coming up with a common DMS take-off threshold in craft beer is much more difficult than anticipated.

6.10 Colour

Lab fermented wort samples, at the end of mini-fermentation were tested for their colour at 430.0 nm. This wavelength measures violet colour. However, it was expected that the colour of the wort would not be very intense as the malt was very lightly kilned in the lab itself (Table 6.10). Also, wort samples were not boiled so no deep colour would develop because of intense boiling.

The objective was to determine if germination temperature had any impact on the colour of fermented wort samples as kilning regime for all the malts was the same. Correlation analysis revealed a correlation coefficient, r = -0.59, and p-value obtained was 0.96. Hence, it was concluded that germination temperature had no impact on wort colour.

Table 6.10: Colour of fermented wort (n = 9)

Temperature (°C)	Absorbance at 430 nm (°SRM)
10.0	16.90
10.0	15.70
10.0	19.00
17.5	10.48
17.5	8.20
17.5	11.91
25.0	9.70
25.0	12.33
25.0	14.90

6.11 Plato Determination

Wort samples were prepared in the lab mainly to collect fermented wort samples for HS-SPME-GC-FID analysis. Additionally, to evaluate the impact of germination temperature on apparent degree of fermentation, apparent extract was measured in degrees of Plato. The data received from mini-fermentation assay was plugged into the logistic model. Speers et al., (141) explained the decline in apparent extract (°P) throughout fermentation by a means of logistic function.

In Table 6.11.1 on the next page, P_i and P_e illustrate initial asymptotic density value and Plato at equilibrium, respectively. B is the rate of consumption, M is the inflection point, RSS is residual sum of squares, OE signifies original extract, AE represents apparent

extract and ADF describes apparent degree of fermentation. Data from nine fermentations was used in the logistic model.

Table 6.11.1: Logistic model parameters for all mini-fermentations (n = 9)

				Density				
Germination Temperature (°C)	**Pi**	**Pe**	**B**	**M**	**RSS**	**OE**	**AE**	**ADF**
10.0	14.81	2.97	-0.12	33.20	3.58	14.60	3.03	0.79
10.0	14.75	1.99	-0.10	33.14	5.18	12.30	2.15	0.85
10.0	14.53	2.09	-0.11	32.14	0.71	14.20	2.16	0.85
17.5	15.01	1.66	-0.11	33.12	1.19	14.70	1.75	0.88
17.5	14.71	1.87	-0.12	28.78	3.61	14.30	1.91	0.86
17.5	14.34	2.21	-0.12	29.00	3.91	13.00	2.25	0.84
25.0	16.38	2.42	-0.09	26.73	27.24	15.13	2.60	0.83
25.0	17.67	0.69	-0.06	28.68	34.40	14.97	1.60	0.89
25.0	16.10	2.10	-0.10	29.52	1.13	15.45	2.09	0.87

Correlation analysis between ADF and germination temperature of malt gave a correlation coefficient, r = 0.48. This coefficient value may suggest an inverse correlation, that with increase in germination temperature, the ADF may reduce. However, Pearson p-value test delivered a p-value = 0.19 i.e., > 0.05. Hence, germination temperature did not have a significant impact on wort ADF.

Wort extract (°P) is the starting density of wort samples without addition of glucose (Table 6.11.2).

Table 6.11.2: Wort extract of all lab wort (n = 9)

Temperature (°C)	Wort Extract (°P)
10.0	11.80
10.0	11.30
10.0	11.20
17.5	11.50
17.5	11.40
17.5	11.50
25.0	11.40
25.0	11.40
25.0	12.00

Correlation analysis between wort extract and germination temperature of malt gave a correlation coefficient, r = 0.29. This coefficient value may indicate a direct correlation, that with increase in germination temperature, the wort extract may increase as well. However, Pearson p-value test delivered a p-value = 0.45 i.e., > 0.05. Hence, germination temperature did not have a significant impact on wort extract.

6.12 Turbidity

Turbidity of wort is conventionally employed to measure premature yeast flocculation (89). However, in this case turbidity readings were taken to see if germination

temperature had any effect on turbidity of wort. A brewing spreadsheet was used to generate these values in Table 6.12, R is the slope, A signifies the absolute amplitude, μ illustrates the midpoint, σ symbolizes the width factor and RSS is residual sum of squares.

Table 6.12: Wort turbidity (n = 9)

			Absorbance (600 nm)		
Germination Temperature (ºC)	**R**	**A**	**μ**	**σ**	**RSS**
10.0	-0.03	2.30	49.51	24.70	2.79
10.0	0.01	1.70	39.48	20.94	0.46
10.0	0.02	1.52	37.85	19.70	0.32
17.5	0.01	1.82	41.01	20.37	0.47
17.5	0.01	1.80	35.64	17.59	0.29
17.5	0.01	1.64	36.00	18.92	1.51
25.0	0.01	1.60	34.00	17.72	1.51
25.0	0.01	1.45	33.74	16.67	1.32
25.0	0.04	1.81	40.40	21.70	0.10

However, the correlation analysis resulted in a correlation coefficient, r = -0.37 but further Pearson's test delivered a p-value = 0.33, which suggested that germination temperature did not have a significant impact on turbidity of wort since p-value > 0.05.

7 Discussion

Two-row barley, as stated by several researchers are preferable over six-row barley because of lower levels of protein and low diastatic power. Two-row barley malts yield 1-2.0% greater theoretical extract (137). According to the results obtained, it was established that the two-row CDC Copeland barley chosen, was perfectly suitable for malting. The barley had exceptional germination energy and capacity in addition to low water sensitivity which indicates that it is capable of vigorous germination, with low dormancy rates and can deal with water stress. Our results were validated with germination results from other research groups like Brower et al., (112).

Numerous methods have been cited in literature review for analysis of DMS and its precursors in malt extracts, fermenting wort and beer. However, preparation of extract by application of heat and its filtration may result in loss of volatiles. The ASBC method Malt-14 (92) an HS-GC method equipped with a sulphur chemiluminescence detector and detects DMSP present in malt extracts. Although, sulphur chemiluminescence detector is specific for sulphur compounds which is best for detection of DMSP but is expensive and wasn't accessible to us. Furthermore, purge and cryotrap methods available in the literature also were reported to have high sensitivity with regards to analysis of DMS and DMSP but they require a special inlet and outlet setup that raised budget concerns along with accessibility issues.

Additionally, Morisaki et al., (93) devised a SPME-GC-FID method for detection of DMSP in soybean seeds directly without any preconcentration or extraction step involved.

Lately, HS-SPME has gained approval as the technique of choice for analysis of highly volatile sulphur compounds in beverages (149). Initially, PDMS and PA fibres were more frequently used ones but because of its strongest affinity for low-molecular-weight sulphur compounds, CAR-PDMS fibre is preferred over others (150, 151, 152, 153, 154).

However, Murray et al., (155) showed that the final amount of analyte adsorbed by CAR-PDMS fibre is largely influenced by unknown third-party compounds along with the concentration of target analyte present in the sample. In the present study the third-party compounds could be thiols. The DVB-CAR-PDMS fibre was more suitable for compounds with higher dissociation constant, and medium to high molecular weight analytes. Studies also established that CAR-PDMS and DVB-CAR-PDMS fibres were hardly different with respect to detection of volatile compounds (159).

Considering the access provided to a Varian 450 GC Chromatograph assembled with FID detector and autosampler, a HS-SPME-GC-FID method was developed by a combination of ASBC Malt-14 and an HS-SPME-GC-FID method by Morisaki et al., (92, 93) to detect DMSP in malt seeds and wort samples directly without any preconcentration or extraction step involved. From a thorough review of literature available, it was determined that 1.0 cm CAR-PDMS fibre was best qualified for detection of DMS and its precursors. However, after calibration of CAR-PDMS fibres, the GC autosampler encountered technical issues between sample runs and splintered two out of three fibres available. Hence, the readily available choice in the lab was 1.0 cm DVB-CAR-PDMS which proved to be just as suitable for our analyses. It should also be noted that all the volatile analyte extractions were done manually, as the GC

autosampler was not functioning, and also to avoid breakage of the rest of fibres available.

Calibration of both HS-SPME fibres was performed according to ASBC method malt 14 (92). Albeit $r^2 = 0.96$, the aberration encountered at standard concentration of 4.0 µg/L compelled us to remove that particular point out of the calibration curve. This complication is not unprecedented as shown by Lopez et al., (144) who experienced notable lack of linearity in response obtained through different standard addition experiments. This could be a result of random errors in the lab and sticky nature of DMS which could possibly be controlled by restriction of concentration range (144). It should be noted that the analytical response is hugely dependent on matrix composition of the sample, which ultimately may lead to complications in correct calibration of the method. It is undeniable that matrix effects are directly related to the concentration of third-party extractable compounds that are a part of the overall sample and are indirectly introduced into the system (144).

A lot of research groups used ethyl methyl sulfide, diethyl ether and several other compounds as internal standards for calibration and sample runs. In correspondence to which, ethyl methyl sulfide was added to our calibration standards as an internal standard for trial calibrations. However, with further delving, studies suggested the existence of competitive effects between analyte and internal standard sulphur compounds for adsorptive sites in the fibre (144). As a consequence of which no internal standard was used for any of our calibrations and sample runs. Additionally, all the samples were coded and randomized in order to avoid bias.

A point to be noticed is that no free DMS was detected by the GC analysis. This could be because without alkali heat treatment of free DMS samples, SMM was not degraded for detection.

Kavanaugh et al., (4) established that DMSP in green malt and DMS of kilned malt are significantly impacted by malting conditions. However, they also emphasized the impact of varietal differences on DMSP and DMS produced by barley with the same index of modification. Varietal impact originated from cultivation conditions of barley such as climate, soil, water quality, temperature and more. These conditions are known to impact total nitrogen content of the grain. Moreover, for the same permanently soluble nitrogen in the extract, index of modification may differ proportionally. A similar index of modification for different barley varieties resulted in variable DMS. Additionally, application of sulphur on barley led to increase in malt diastatic power and furthered the concentration of SMM in kilned malt (134). Two-row CDC Copeland barley used for this study was grown on a farm in Saskatchewan (MB) and the climatic conditions of the field, water quality, soil and temperature will have an undeniable impact on the total nitrogen of the grain, which ultimately will impact the levels of DMSP evolved in the malt. As mentioned above, that similar index of modification resulted in different DMS levels in different barley varieties, there could be a possibility of this transitioning unto the seed level. As the lab-scale germination was not a strictly controlled process, all seeds had a variable index of modification, which possibly resulted in variable levels of total and free DMS between replicates.

In fermented wort obtained from kilned malt samples, no DMSP was detected. Possibly, the majority DMSP was degraded during fermentation, as suggested by a study in

brewery-scale fermentations, a 64 to 72% reduction in DMS with a mean of 69% in nine fermentations was observed (140). If this happened with our mini-fermentations, the remainder DMSP would be lower than detection limits of our method. Secondly, autoclaving of wort and the filtration possibly impacted the chances of DMSP generation in the wort samples.

DMSP concentrations measured in pneumatic malt were 3 times the DMSP levels in floor malt suggesting that germination temperature may not be the primary cause of the origination of DMSP. Contrary to the hypothesis that floor malt will have higher DMSP levels than pneumatic malt. However, a study with a much larger sample size, more efficient equipment and different varieties of barley both floor and pneumatically malted would be required to establish this particular hypothesis.

Detected amounts of total and free DMS are hugely dependent on matrix of malt seeds and the other solutions such as sodium hydroxide added in the vial, which may influence the accurate determination of DMSP by competing with the target analyte for adsorption on the fibre (145).

The volume of samples has been decreased successively in a parallel manner to the increase sensitivity of detectors. For detection of volatile sulphur compounds in wine, few research groups sampled 25.0 mL of wine (151, 149, 153). Over time, the sample volume was reduced to 10.0 or 15.0 mL (150, 154) and the conventional methods proposed an optimal wine sample volume of 5 mL. Reduction in wine sample volume from 25.0 to 5.0 mL increased the sensitivity of the pulsed flame photometric detector. Additionally, this detector is relatively economic and straightforward to use than

sulphur chemiluminescence and atomic emission detectors (156). In our study the sample volume was 9 mL and 8 mL for total and free DMS vials respectively with 1-1.10 g of malt in each. However, keeping amount of malt the same and reducing the volume of solutions added to the vials to 5.0 mL may obtain a relatively linear response.

Despite the reduction in sample volume, matrix effects are nonetheless conspicuous which eventuates change of internal standards and calibration strategies to alleviate the problem (151, 146, 152, 153) however, these issues cannot be completely overcome. The total and free DMS samples were also tested on the GC-MS to detect other compounds eluting, it was observed that thiols eluted right after DMS. Which may also add to the variation in the results obtained as thiols may compete with DMS for adsorption on the fibre.

Lopez et al., (144) executed all analysis using Varian CP-3800 gas chromatograph fitted with a pulse flame photometric detector which was fitted with DB-Waxetr 30m × 0.32mm internal diameter with 1 μm film thickness to detect DMS in wine samples. The temperature program used by them was not very different from the temperature program followed for the present experimentation, which may suggest that the method used for the detection of DMSP may not have major flaws.

Wardencki (145) found that peak areas for DMS varied by 15% in both wine and synthetic solutions. He speculated that the atmospheric oxygen present in the vials caused poor repeatability and after flushing the vials with nitrogen before introduction of wine samples, found that variation reduced below 15%, hence, the repeatability improved markedly. A similar precautionary measure was suggested by Majcenovic et

al., for analysis of DMS (151). Screw top vials with teflon septum were used for sampling all malt and wort samples. However tightly screwed, there is contingency of atmospheric oxygen entering the headspace of vials that may have resulted in poor repeatability or variability among the sample runs. If crimp top vials were used, along with flushing the vials with nitrogen before introduction of all our samples, better reproducibility or less variation may have been achieved.

Murray (155) illustrated that CAR-PDMS fibres have limited adsorption sites and during analysis of volatile sulphur compounds, the compounds with low affinity for those sites are readily replaced by others with higher affinity. While DVB-CAR-PDMS fibres have more affinity towards medium to high molecular weight compounds. But both fibres were just as suitable for analysis of volatile flavour compounds. For volatile sulphur compounds such as DMS, an extraction time of 20.0 min or longer will further result in higher extraction of sulphur compounds. But because of thermal instability of and feasibility of experimentation, the extraction time was set for 20.0 min. However, for this study, several extraction or adsorption times and temperature were tested before choosing 30.0 min at 40.0^{o}C. First the samples were incubated at 25.0^{o}C for 10.0 min as suggested by Morisaki et al., (93) for detection of DMSP in soybean seeds, but not a lot of DMS was detected in our malt samples, which could be credited to the change in sample matrix. Secondly, the samples were incubated at 30.0^{o}C for 20.0 min which seemed to improve the extraction. As DMS has a boiling point of 38.0^{o}C, the samples were incubated at 40.0^{o}C for 30.0 and 40.0 min which resulted in more resolved peaks and better repeatability.

For the actual runs, an adsorption time of 40.0 min at 40.0ºC was selected. Unfortunately, after a few runs the GC autosampler underwent technical issues and started to malfunction which resulted in broken fibres as mentioned above. Along with broken fibres, the autosampler was unworkable, which also meant that all the sampling and injections were to be done manually. This introduced time constraints as the sampling wouldn't be continued automatically. To tackle these issues and perform all the sampling within contract limit with the lab facility, the incubation time was dropped down to 30.0 min at 40.0ºC.

Muller et al., (115) communicated that increase in germination temperature by 1.0ºC resulted in an increase in malting losses by 0.3% and lowered extract yield by 0.4%. In contrast, the present study suggests that germination temperature of lab floor malts had no significant impact on wort extract and apparent degree of fermentation. Additionally, germination temperature had no significant impact on wort colour and turbidity. However, a correlation analysis was run for all mini-fermentation parameters and wort colour (Table A.1, Section 9) which suggested a significant relationship between germination temperature and initial asymptotic density of the wort (P_i). The analysis also suggested significant relationships between other factors of fermentation exclusive of the germination temperature, e.g., apparent extract of wort was significantly correlated with density at equilibrium and germination temperature was significantly correlated with the inflection point of the logistic curve.

During the lab-scale malting, a standard operating protocol was developed for production of true replicates. However, if resources weren't a limiting factor, a micro-malting machine for lab-scale floor malting would have resulted in more uniform lab

floor malt, possibly with less heterogeneity. Installation of a purge and cryotrap system would prevent the loss of DMS during extraction and transfer onto the column, along with a GC sulphur chemiluminescence detector or a mass spectrometer with some changes in the method would increase uniformity of the results. An autosampler is beneficial in terms of its accurate timing and ability to inject the sample onto the column without any third-party contact and delay. If time wasn't a constraint more samples would have been tested manually in order to attain uniformity. However, with the method used for our experimentation, there is no surety of excellent repeatability. Hence, in order to get reproducible results in malt seeds directly, a novel method that counteracts the third-party interference during sampling should be developed.

These field sensory studies did not corroborate with our GC results as shown above. Furthermore, the evaluation of DMS-take off threshold in floor malt APA was inconclusive. This inability of the participants to detect DMS in floor malt beer could be justified by the low levels of DMSP obtained in floor malt, Rosenthal effect, majority of panelists fell under the "low sensitivity" category or were DMS insensitive. This did not support hypothesis that beer made from floor malt may have high DMS levels due to which DMS flavour threshold in this particular beer would be high as well. Another major factor that may contribute to this result was overall sensory fatigue of the participants. However, there is little information in literature on the effects of sensory fatigue, of motivation, and of effects on endogenous concentration. Lastly, there is no universally accepted criterion of what constitutes a threshold concentration (104), which only makes threshold evaluation of DMS in craft beer more perplexing. In the future, sensory evaluation for DMS might be done in different types of beers made neutral base malts and lower hopping rates to avoid overwhelming the participants.

8 Conclusions and Future Work

Several factors were thought to induce high levels of DMSP, but only a few have been tested. Two-row barley has been known to give lower levels of protein which also leads in lower production of SMM which is the inactive form of DMSP (113). The germination attributes of two-row CDC Copeland barley were satisfactory for malting purposes.

Green malts did show that malts germinated at higher temperature generated greater levels of DMSP, as supposed by Kavanaugh et al., (4). However, post kilning, this trend did not follow through and malts germinated at 25.0^{o}C had the lowest levels of DMSP. Whereas, fermented wort samples produced from malt samples germinated at 10.0, 17.5 and 25.0^{o}C did not have detectable levels of DMSP. The present results suggested that kilning at 63.0^{o}C for 24.0 hours did not have a significant reduction in DMSP levels. Additionally, germination temperature had no significant impact on wort extract, apparent degree of fermentation, wort colour, turbidity.

Further, detection of DMSP in floor and pneumatic malt revealed that two malting treatments had a significantly different impact on DMSP levels generated.

Lab floor malt and commercial floor malt results collectively did not support the hypothesis that floor malt germination temperatures result in greater DMSP levels. Sensory analyses did not support the second hypothesis that floor malt beers will have higher thresholds for DMS.

From the results obtained, it could be concluded that floor malt is not inferior to pneumatic malt with respect to origination of DMSP and is perfectly suitable for brewing purposes without the concern of incurring energy and processing costs for off-flavour development in beer.

However, another arena that could be probed further would be spraying of nitrogen and sulphur during growth of barley crops, and floor germination time and kilning regimes with respect to generation of DMSP.

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Appendix

Table A.1: Correlation analysis for all Fermentation Parameters (n = 9)

	Temperature	*Pi*	*Pe*	*B*	*M*	*RSS*	*OE*	*AE*	*ADF*	*R*	*A*	*μ*	*s*	*RSS*	*Colour*	*Wort Extract*
Temperature	1															
Pi	0.786892641	1														
Pe	-0.43616528	-0.576	1													
B	0.584242658	0.9119	-0.6924	1												
M	-0.80228143	-0.5308	0.2072	-0.4071	1											
RSS	0.611038473	0.85073	-0.4842	0.89239	-0.607	1										
OE	0.627217538	0.61238	-0.1073	0.28744	-0.358	0.32675	1									
AE	-0.3600397	-0.306	0.9133	-0.401	0.1265	-0.152	-0.0277	1								
ADF	0.479209676	0.42571	-0.9141	0.47875	-0.193	0.22272	0.16095	-0.9856	1							
R	0.126215416	0.19962	-0.564	0.47255	-0.287	0.37692	-0.2481	-0.5203	0.4819	1						
A	-0.37327338	-0.3701	0.6502	-0.5785	0.4633	-0.4727	0.0966	0.61415	-0.609	-0.9631	1					
μ	-0.55559674	-0.3894	0.5903	-0.5143	0.7414	-0.5465	0.0199	0.57449	-0.579	-0.7919	0.89208	1				
s	-0.5374535	-0.4144	0.6614	-0.5247	0.7105	-0.6031	-0.0544	0.61346	-0.609	-0.7285	0.81843	0.96323	1			
RSS	-0.10493965	0.08278	0.4149	0.01126	-0.016	0.31461	0.06069	0.69543	-0.696	-0.3895	0.46163	0.39584	0.304833	1		
Colour	-0.58879201	-0.2243	0.2944	-0.1297	0.6075	-0.3377	-0.1824	0.35176	-0.375	0.01917	0.1469	0.52299	0.619294	0.08377	1	
Wort Extract	0.288675135	0.13717	0.3369	-0.2074	0.0057	-0.2435	0.45991	0.30903	-0.224	-0.7608	0.63505	0.56025	0.600424	0.22192	0.09142	1

The values in the table above represent p-value for each factor listed on the left. These p-values were derived in order to delineate their impact on the DMSP produced in the malt. However, only the factors with p-value above 0.67 were found to be significant as highlighted in yellow.