Investigating the Influence of the Extent of the Malting Process of *Hordeum vulgare* on the Growth of *Saccharomyces cerevisiae* in Liquid Culture

1. Introduction

In this exploration, I explore how the extent of malting grain affects the growth of fungi in liquid cultures. The fungal kingdom is often ignored in our society despite the massive role it plays in many industries, such as the culinary, pharmaceutical (Mycorena), waste management (Low), and even the packaging industry (Southey). The kingdom itself is often misunderstood to the point that a 2013 study found that the majority of Turkish teachers-in-training thought that mushrooms were plants (Yangin 184). Regardless, fungi are fascinating and adaptable organisms. While most fungi evolved to grow on trees, many fungi can grow on multiple mediums from sawdust, coffee grounds and even in liquids–the focus of this exploration (Shields). The specific idea that fungi can grow in liquids is something that confused me for a very long time, and I explored how the process works. It is called liquid culture and requires the fungi to grow on a sterile, nutrient-rich medium that is often made from plants that have undergone a special process called malting ("Mycology Lab Skills: Plates, Slants, and Liquid Culture"). Malting is used to produce one of the oldest beverages on the planet, beer, with a combination of a barley (*Hordeum vulgare*) malt, and the fungus *Saccharomyces cerevisiae.* One of the most interesting parts of this process is malting itself as it converts simple grains into a nutrient suitable for the industrial production of liquid cultures. In contrast to most modern food processing, malting has existed for millennia (Vinje et al. 195). To explore this complex bridge between botany and mycology using the scientific method, I investigated how much malting is required to gain its growth-boosting benefits when used in fungal liquid cultures.

1.1 Background Information

Germination, in general, is the process of a seed beginning to grow upon its environment being conducive to plant growth. This is a natural mechanism of seeds that allows them to only grow after seed dispersal into the right environment and after the weather and season allow for eventual plant maturity. The basic requirements for germination are moisture, heat and oxygen, all of which allow metabolism to take place. The metabolic rate of a cool, dry seed is almost zero because metabolic reactions are enzyme-catalyzed. Enzyme catalysis must occur in the aqueous cytoplasm, lumen and matrices of cells, chloroplasts and mitochondria respectively. Aerobic cellular respiration cannot occur without oxygen, while photosynthesis is impossible without water. Shortly after these conditions are met, the hormone gibberellin is produced. Gibberellin stimulates mitosis in the embryo of the seed and triggers the expression of the genes responsible for the production of various enzymes. Of these enzymes, α-amylase is one of significant focus. It is produced in the embryo and migrates to the starch reserves of the seed, hydrolyses the bonds of the starch granules and produces maltose. α-Glucosidase catalyzes multiple saccharides, but most often will catalyze the conversion of maltose to two glucose molecules (Vinje et al. 195). Other enzymes, such as ß-amylase, are produced during seed development while the seed is still on the parent plant and are released in the first days of germination. ß-amylase performs a very similar role as α-amylase, hydrolyzing starch bonds and creating maltose that is broken down in subsequent reactions. ß-amylase levels are a significant contributor to the enzymatic activity of a seed (Vinje et al. 196).

Malting is a form of germination, but the process of germination with the intent of growing a seedling and the process of malting is very different. Malting is a very complex process relative to conventional germination, known herein as simply germination. In conventional germination, a seed is given access to moisture, often by soaking, and left in a humid environment to germinate. To malt a seed, it is soaked for a comparatively large amount of time in cycles of submersion and aeration until a specific moisture content is reached. The seeds are then allowed to germinate under specific conditions and agitated frequently. Malting also contains a kilning step to halt growth and create a stable product (Vinje et al. 195-196). Malting a grain before using it for alcohol production increases carbohydrate availability, desirable flavour, and the efficiency of the yeast in producing alcohol, hence it has been in use for thousands of years by maltsters to create better malts for better beverages (Vinje et al. 195).

For any organism, specifically *Saccharomyces cerevisiae*, to grow, it must perform the seven functions of life: nutrition, metabolism, growth, response, excretion, homeostasis and reproduction. Of particular importance to one who wants to control the growth of a culture of yeast are the functions of nutrition and excretion. These can be controlled by adjusting nutrient levels in the culture, thus allowing the excretions of the yeast culture to be

removed and controlling environmental parameters such as temperature and osmolarity. The process of brewing beer is essentially making a liquid culture of yeast, often *Saccharomyces cerevisiae,* in a medium of *Hordeum vulgare* malt products. In a simplified beer fermentation process (referring to the biotechnology term for a culture, often liquid, producing a metabolite of interest), a *Hordeum vulgare* malt is combined with hot water, resulting in a medium called "wort." The wort contains the nutrients necessary for *Saccharomyces cerevisiae* to live, while excretion is carefully managed to produce ethanol as a metabolite. Flavour profiles and the other functions of life are more often controlled by the variety of *Saccharomyces cerevisiae* and its unique responses to the stimuli of the wort environment (Lodolo et al. 1018-1020). In beer fermentation, *Saccharomyces cerevisiae* colonies eventually perish due to a lethal concentration of ethanol excrement from anaerobic respiration (another, unique, process that happens to share the name of fermentation), caused by a deprivation of oxygen to the yeast cells. However, other liquid cultures that do not do this can also be created using media of *Hordeum vulgare* malt-based worts (Spencer and Spencer). This allows aerobic respiration, a more efficient metabolic pathway to create ATP, to occur and hence increase the growth of the *Saccharomyces cerevisiae*. *Hordeum vulgare* malt-based worts provide the essential nutrients for yeast growth, organic carbon, in the accessible form of mono and disaccharides, nitrogen in numerous forms including amino acids and free amino nitrogen (O'Connor‑Cox and Ingledew), as well as various other trace elements, hormones, proteins and other compounds (Berry and Brown, ch.6).

Fungal growth in a liquid culture cannot be directly observed, however, the amount of aerobic respiration and therefore energy consumption of the yeast culture may be measured by the magnitude of the decrease in the mass of a sample (Koutsokali and Valahas 2). It may also be measured by the decrease in specific gravity of the medium, measured before and after yeast growth. This is because specific gravity measures the relative density of water and hence the mass of dissolved compounds, which, in the case of a wort-like liquid culture are mostly sugars and amino acids. The specific gravity may also be used to estimate the growth potential of the fungus in the media (Hardwick). The viability of a *Saccharomyces cerevisiae* liquid culture can be measured by the rate of gas production, measured by pressure changes, as a result of respiration (in yeast, both metabolic pathways result in the excrement of gaseous carbon dioxide) when a new sugar source is introduced to the culture (Milani et al. 44).

1.2 Research Question

What is the effect of the extent of the malting process followed for *Hordeum vulgare* on the growth of *Saccharomyces cerevisiae* in a *Hordeum vulgare* malt-based liquid culture medium?

1.3 Hypothesis

H_1: The amount of growth of *Saccharomyces cerevisiae* in a *Hordeum vulgare* malt-based liquid medium increases with the extent of the malting of the *Hordeum vulgare.*

H_0: The amount of growth of *Saccharomyces cerevisiae* in a *Hordeum vulgare* malt-based liquid medium does not change with the extent of the malting of the *Hordeum vulgare.*

1.4 Variables

1.4.1 Independent Variable

The extent of the malting process of *Hordeum vulgare,* specifically the soaking and germination portions. This variable ranges from the control, no malting at all, and a complete malting process, described in the method of this exploration.

1.4.2 Dependent Variables

The growth of the *Saccharomyces cerevisiae.* This cannot be directly measured, therefore the following properties are measured: the visible properties of the media, the specific gravities, and the ratios of wet to dry weight. In this exploration, the values will be compared from before and after the inoculation and growth of the *Saccharomyces cerevisiae* of the same base *Hordeum vulgare* malt of the media, as well as between the different *Hordeum vulgare* malt groups. For the wet-to-dry mass ratios, a decrease in the ratio of dry mass to wet mass indicates *Saccharomyces cerevisiae* growth (Koutsokali and Valahas 2). A decrease in the specific gravity of the

media indicates *Saccharomyces cerevisiae* growth (Hardwick). Increased pressures during a viability test indicate higher levels of active *Saccharomyces cerevisiae* in a given sample (Milani et al. 44).

1.4.3 Controlled Variables

Table 1: *Controlled variables, explanations, and methods of control.*

Variable	Explanation	Method of control
Environmental temperature	Different temperatures affect the rate of seed germination by affecting the rate of reactions in enzyme-catalyzed metabolic pathways and by the potential of denaturing enzymes and proteins in the same pathways due to excess heat (Hardwick).	All *Hordeum vulgare* seeds are from the same source. All malting variables are controlled by the manner of keeping all samples together in the same environment, undergoing the same procedures at the same time such that any variable would equally affect the malt groups. All procedures were performed at the same time with the same equipment.
Atmosphere	Different atmospheric compositions of CO_2, O_2 and H_2O in the local atmosphere during malting and yeast growth will affect the water content of the organisms as well as the amount of aerobic respiration that can occur in the organisms.	
Grinding Size	Smaller grinding sizes allow faster enzyme release and faster starch decomposition due to the effect of higher surface area-to-volume ratios at small grind sizes.	
Hordeum vulgare cultivar	Different cultivars and gene expression *in planta* can unpredictably affect the viability and unique germination parameters of the *Hordeum vulgare* seeds.	
Dehydration of malt	The dehydration of the malt is responsible for halting germination without denaturing enzymes, the temperature and time of dehydration will affect the final malt moisture and the extent of metabolic deactivation in the malt. Higher temperatures and longer dehydration times increase the risk of enzyme denaturing (which reduces the amount of starch conversion) but also increase the shelf stability and metabolic deactivation of the malt (Vinje et al.).	
Saccharomyces cerevisiae source	Different sources of *Saccharomyces cerevisiae* may have different plasmids, a form of non-genomic DNA often shared between yeast cells in culture that can unpredictably affect metabolic and growth characteristics of the inoculated culture.	The source of *Saccharomyces cerevisiae* is kept constant by using the same jar of yeast and by homogenizing the source by creating a starter before inoculation. All procedures were done in identical conditions at the same time with identical procedures such that any change in a variable had an equal effect on all cultures. All reactors were created identically.
Malt steeping	The malt steeping temperature and time are both positively correlated with increased enzymatic activity, although extremely high temperatures can result in the denaturing of enzymes essential to an ideal steep (Hardwick).	
Inoculation temperature	The inoculation temperature of the yeast must be kept in a temperature range that will not shock, or even kill the yeast starter.	
Water source	Different water sources have varying amounts of dissolved compounds, including gasses, essential minerals, biocides and metals. These concentrations can often unpredictably vary with each water source, and therefore unpredictably affect growth in a liquid culture (Hardwick).	
Reactor parameters	Reactor size, thermal conductivity, light permeability, material, and airflow will each affect the growth of a liquid culture in numerous ways (Berry and Brown).	
Testing equipment	Equipment may be incorrectly calibrated and give inaccurate results, even if precision is not affected.	All testing was done in the same environment at the same time with the same equipment. This means that although inaccuracy is possible, it will not affect the results, as they are a measure of the differences between measurements and therefore irrespective of accuracy.
Testing environment	The testing environment, including temperature and atmospheric conditions, can affect how samples react to different tests and the environment-dependent calibrations of testing equipment. All of these variables possibly affect the accuracy but not the precision of the measurements.	
Dehydrating of media samples	The dehydration of the samples can occur at varying rates, and even the oxidation and hence mass of the samples.	All dehydration was done simultaneously as defined in the procedure, therefore affecting all samples equivocally.

2. Procedure

2.1 Materials

- 5kg *Hordeum vulgare* (barley) seeds
- 60g Fleischmann's® Active Dry Traditional Baker's Yeast (*Saccharomyces cerevisiae*)
- Tap water
- Paper towel roll
- 100g pure white sugar granules
- 29 Bernardin® 86mm SNAP LIDS® discs
- 20mL Star San™ Concentrate
- Soap
- 1m 3M® Transpore™ surgical tape
- 50cm by 30cm sheet of aluminum foil
- Reverse osmosis water with restored minerals
- Machining Oil

2.2 Apparatus

- Excalibur Econ 2900 9-tray food dehydrator
- 2 large (approximately 24-quart) steel pots
- 10-quart plastic mesh bag
- 5 freezer bags, one-gallon capacity
- Laptop Computer
- Kitchen measuring containers:
  - 500mL±25mL
  - 2L±0.25L
  - 2-teaspoon measuring spoon (no uncertainty provided)
- Electronic thermometer ±0.1°C
- One 100mL±0.5mL graduated cylinder
- Five 30mL±0.25mL graduated cylinders
- One 200mL Eyrlenmeyer flask
- Vernier Gas Pressure Sensor (±4kPa)
- Lab oven (660 watt)
- Samsung A51 smartphone
- Two 18 by 12-inch plastic containers with a depth of approximately 5cm
- Dual-burr malt grinder
- 5 deli-style 300mL containers
- Sharpie
- Knife
- Large rubber storage bin
- Hydrometer (±0.005 units) with matching hydrometry cylinder
- 29 Benardin® 750mL Wide-Mouth Jars
- 29 Benardin® 86mm SNAP LID® rings
- 3/8th inch metal-cutting drill bit
- Drill press
- Lab scale (±0.001g)

2.3 Method

Method for Malting *Hordeum vulgare*

The uses of malted grains are vastly different from those of germinated grains, and thus the processes are very different. This malting process comes from an interview with a local maltster and supplier of the *Hordeum vulgare* seeds used in this exploration, Devin Hussman. His process includes a soaking, germinating, and dehydrating process, as detailed below in the context of the exploration.

5 groups of 1000g±1g of *Hordeum vulgare* seeds are poured into 5 freezer bags (gallon-sized), labelled G1 (Group 0) through G5 (Group 5). G1 is immediately removed from the freezer bag and spread evenly into a household food dehydrator (Excalibur Econ 2900) and dehydrated at 50°C for 24 hours. G2 through G5 are placed in a large rubber bin and filled with reverse osmosis water until the water level is approximately 5cm above the *Hordeum vulgare* seeds, thus beginning the soaking portion of the malting process. The bags are agitated to remove air pockets and allow any floating debris to surface, at which point the debris is removed and discarded. The *Hordeum vulgare* seeds are then allowed to soak for approximately 5 hours, after which about 30 slits each measuring 2-5mm long are carefully cut into each bag to allow all liquid to escape and to be discarded. The tops of the bags are then opened to allow airflow to evaporate excess water and prevent the unintended growth of mould. The bags are left like this for 19 hours. After this, G1 is removed from the dehydrator, placed in its bag and refrigerated to maintain the chemical state of the *Hordeum vulgare* and prevent contamination. G2 is removed from its bag and dehydrated in the same manner as G1. G3 through G5 are once again soaked by filling the rubber bin they are placed in with reverse osmosis water until the water level is approximately 5cm above the surface of the *Hordeum vulgare* seeds, the bags are again agitated to remove air pockets and redistribute the seeds within the variable-moisture microclimates of the bags. They are left for 5 hours to soak, and once that is complete, the liquid is discarded through the holes made in the last cycle. The tops of the bags are once again opened to allow for the evaporation of excess moisture. After another 19 hours, G2 has finished the dehydration process and is stored identically to G1. G3 is then dehydrated identically to the previous groups. G4 and G5 now begin the germination portion of the malting process. They are placed in 18 by 12-inch plastic containers with a depth of approximately 5cm of depth. Then they are gently stirred with

washed, bare hands to minimize the effects of varying moistures in the microclimates, and a large piece of paper towel is placed atop the containers to prevent excess evaporation and environmental contamination. After 24 hours, G3 is removed from the dehydrator and stored identically to the previous groups. The paper towels are removed from the containers containing G4 and G5, and the germinating seeds are stirred again with washed hands to separate clumps of seeds and minimize the effect of the varying moistures of the microclimates within the containers. The containers are then placed under a large, inverted and clean rubber storage bin, over a carpeted surface to allow for air circulation without excess moisture losses. After 48 hours, and a total of 72 hours of germination, G4 is dehydrated and later stored identically to the previous groups. G5 is left alone for another 24 hours, for a total of 96 hours of germination and a completed malting process. It too is dehydrated and stored identically to the previous groups.

Creating Liquid Cultures

This method for creating liquid cultures is inspired by papers, books, and websites by Vinje et al., Hardwick and "Mycology Lab Skills: Plates, Slants, and Liquid Culture," as well as the equipment available and financially feasible during the exploration.

The malted *Hordeum vulgare* groups are ground in a dual cylindrical-burr grain grinder with the distance between the burrs measuring 0.4mm±0.1mm. Each group is ground with identical settings and the grinder is cleaned between batches. The malt groups are each weighed to find their weights after their various malting processes.

Liquid culture reactors are made using 29 Benardin® 750mL Wide-Mouth Jars with 29 sets of two-part, 86mm SNAP LIDS®. The disc portion of the SNAP LIDS® each has an off-centred hole cut into it using an oiled metal 3/8th-inch drill bit and a drill press. The lids are then post-processed to remove sharp edges using sandpaper and tin snips. Each lid is cleaned in a bath of hot, soapy water to remove any oil from the cutting process. The jars and lid components are all cleaned of debris in hot, soapy water, and then made sterile using an acid-based Star San® no-rinse sterilization solution made according to the manufacturer's instructions. The components are kept sterile this way until they are immediately necessary to the procedure.

To produce the liquid culture, a large pot containing 6.0L±0.5L reverse osmosis water is heated to 75°C and a gas stovetop and an entire group of ground, malted *Hordeum vulgare* is placed into a mesh bag. The mesh bag is placed into the pot of water, stirred to break apart dry clumps, and the temperature is manually maintained at 75°C for 30 minutes, at which point 10g±1g of pure white sugar (sucrose) is dissolved in the solution. After that, the bag containing the malt is lifted out of the liquid and massaged for approximately 10 minutes to remove the majority of the liquid from the malt bag. The liquid is passed through a mesh filter to remove any large particles. Seven batches of 300ml±25ml of the liquid are then made, 6 of which are poured into the liquid culture reactors, and the seventh is placed in a plastic deli container, labelled according to the malt used, for later testing. These frozen samples will be known as the control samples from now on. For the liquid culture reactors, all components of the reactor are removed from the sterilization solutions. The lids are placed on the jars and approximately 2cm by 3cm strips of 3M® Transpore™ surgical tape is placed atop each of the holes in the lids. The jars are labelled G1 through G5 according to the malt that is produced in the liquid culture used. A layer of household aluminum foil, cut to cover the lids of the jars, is placed and crimped over the lids to prevent airborne contamination and to prepare for heat sterilization. The deli container and its contents are frozen to prevent changes in the sample from occurring and to prevent the growth of any contamination. Note that G1 only has 5 liquid culture containers made, instead of 6, due to an unlikely event of human error. All equipment is cleaned between batches, with the excess liquid poured down a drain into a septic system that can break down the liquid. The used malt is also disposed of via a garden composting system. This is repeated for each group.

While this is happening, the finished jars covered in foil are heat-sterilized to eliminate any biological contamination contained in the malt-based liquid medium. This is done because no sterilization has been possible during the malting process as no proteins or enzymes could be denatured so far, now that the malting has finished, sterilization can finally occur. In batches of five liquid culture reactors, approximately 500mL of tap water is poured into a large boiling pot and the reactors are placed inside. The pot is covered with a lid, and the water inside is brought to a boil lasting 30 minutes via a gas stovetop, after which the heat is removed along

with the lid. Once the jars cool to a comfortable handling temperature, they are placed in a large bath of water for storage until the rest of the jars have gone through this process. This allows all reactors to reach the same temperature over time and controls the inoculation temperature. Once this point is reached, and all jars are at an equal temperature, inoculation may occur. At some point before the end of this portion of the process, the *Saccharomyces cerevisiae* starter may be created.

A *Saccharomyces cerevisiae* starter is made by pouring 294g±2g of boiling-hot reverse osmosis water into a container sterilized with Star San® and 35g±1g of pure white sugar that is to be dissolved. The solution is allowed to cool to approximately 40°C. 35g±1g, at which point 54g±1g of Fleischmann's® Active Dry Traditional Baker's Yeast (*Saccharomyces cerevisiae*), previously stored in a refrigerator as per the manufacturer's instructions, is added to the solution and stirred. The solution is left for approximately one hour with frequent stirring to reduce foam.

For the inoculation, each reactor has its aluminum foil removed and the lid unscrewed and removed using the foil to minimize contact with the lid. 2 teaspoons of the *Saccharomyces cerevisiae* starter is added to each reactor. The lid is placed back on the reactor and the aluminum foil is disposed of in a household garbage bin. This is done quickly and carefully, with washed hands, to minimize contamination risk from airborne and contact-spread organisms. The reactors are collected together in a large rubber storage container recently cleaned with soap and water. Once all of the reactors have been inoculated and placed in the bin, the lid of the container is loosely placed on top to allow airflow without excess risk of contamination from falling particles. The bin is placed in a cool, dark place for 5 days to grow the *Saccharomyces cerevisiae* colonies.

Testing

Once the reactors have finished growth, they are ready to be tested. All tests are conducted with clean hands and equipment, and the medium is always agitated to temporarily deflocculate the sediment to gain an accurate sample.

The first test is a specific gravity measurement using an analogue hydrometer with a precision of ±0.005 on the 60F scale. Each reactor has a sample poured into the hydrometer flask. A reading is taken after spinning the hydrometer to remove any adhered air bubbles and allow the meter to settle. The resulting measurement is recorded. This is also done to the control samples of the original media after they are thawed to room temperature. Note that this test cannot be accomplished on groups G1 and G2 due to their viscosity, and it is not possible to gain similarly accurate results with other methods, resulting in the loss of some data points.

The viability test is done by taking an average sample of each media group, not including the uncolonized control media. To do this, a pressure sensor is used to measure to pressure of the gaseous products of *Saccharomyces cerevisiae*'s aerobic respiration of glucose. This is done by taking an equal sample of medium from each media within a group until the total amount of media is 25.00mL±0.25mL, using a pipette and a graduated cylinder. In this case, 4.17mL±0.25mL from each reactor in groups G2 through G5, and 5.00mL±0.25mL from each reactor in G1. Note that the samples from different groups must be kept separate, meaning each group's sample is kept in a different graduated cylinder. Before testing the viability of the sample, a solution of 100.0mL±0.5mL of tap water with 10.000g±0.001g of pure white sugar dissolved in it is created. This is then transferred into an Erlynmeyer flask compatible with the Vernier Gas Pressure Sensor stopper. The average sample previously made is quickly poured into the solution and stirred to distribute the *Saccharomyces cerevisiae* cells and aerate the solution. The pressure sensor attached to the rubber stopper is pressed onto the flask and the valve is open to equalize the pressure. The valve is closed and the data collection begins, lasting 200 seconds. The initial pressure and final pressure are recorded. This is repeated for each average sample of the groups. The average sampling method gives an additional source of data while not taking an excessive amount of time to complete, hence it is used over completing this test 29 times.

Next, the ratio of the wet-dry masses of the media is taken. 35 small crucibles are weighed before and after adding a small amount of each liquid medium (from the unused control media and the used media) until the liquid level is approximately 7mm below the lip of the crucible. This prevents losses from boiling liquids splashing out of the crucible. The crucibles are placed in a tabletop lab oven. The temperature is raised to the

maximum setting for 2 hours and then turned off for 20 hours to allow for natural evaporation. The oven is then turned back on at the highest heat setting until the samples are visibly crisp and dry, approximately one hour. The weights of the crucibles are taken once again.

The last test is a qualitative measurement of the visual differences between the unused and frozen reactor contents (the original liquid culture medium), and the used media containing the *Saccharomyces cerevisiae* colonies. The media are also visually compared to each other. First, a used media sample from each group (G1 through G5) is selected at random, which is acceptable in this specific case for a qualitative visual test because the media within each group are visually identical. A photo is taken at automatic exposure settings with a Samsung A51 smartphone, the contents of the jars are disposed of as previously described, and washed until the jars are clear. The contents of the original media samples are poured into the same jars, which are identical, therefore controlling the potentially variable jar designs. Another picture is taken in the same way from a similar point of view.

2.4 Risk Assessment

Safety:

Hot liquids, flames, sterilization solutions, and biological cultures are all used in this exploration. Appropriate education on the methods of safe use for all equipment occurred, along with all procedures taking place with close access to first aid kits, fire extinguishers, and cold water for potential burns. The sterilization chemical used was specifically chosen for its reduced need for safety precautions compared to other options. In the event of biological, specifically bacterial, contamination, it would have happened in a sealed reactor that could be easily disposed of in a low-risk manner without exposing the reactor to open air. Reactors were carefully checked for a lack of contamination before opening for testing. Lab personnel were trained and certified in emergency first aid.

Environmental:

All materials used in this exploration pose no environmental concerns as can easily be disposed of down a household drain or be composted in a garden. No invasive species of any organism were used.

Ethical:

There are no ethical concerns to consider for this exploration.

3. Observations

3.1 Quantitative Data

Table 2: *Specific gravity measurements (±0.005 on the 60F scale). Note that G1 and G2 were not tested due to their physical properties and that the non-control samples were taken from different reactors. Also, note that the identification numbers on the left-hand side are not consistent between tests.*

	G3	G4	G5
1	1.022	1.024	1.019
2	1.021	1.023	1.021
3	1.020	1.026	1.020
4	1.022	1.027	1.020
5	1.021	1.023	1.021
6	1.022	1.024	1.021
Control	1.025	1.035	1.037

Table 3: *Raw data for the wet-dry ratio measurements (in g±0.005g). Note that the identification numbers on the left-hand side are not consistent between tests. The rest of the table is continued in Table A.3*

	Empty Crucible	Wet Crucible	Dry Crucible
G1			
1	7.781	12.19	8.001
2	9.803	*16.202*	*10.129*
3	11.015	15.182	11.227
4	*7.025*	11.849	7.276
5	*8.946*	13.313	9.169
Control	16.974	27.443	17.762
Continued in Table A.3			

Table 4: *Pressure data, measured in kPa±4kPa. The pressures are measured from the initial pressures (indicated as such), and the pressure after 200 seconds (marked as the final pressure).*

	G1	G2	G3	G4	G5
Initial	102	102	102	102	102
Final	103	103	102	101	102

3.2 Qualitative Data

During the malting phase of the procedure, the soaking liquid gained a tan colour throughout the soaking. The scent of the *Hordeum vulgare*, a slightly funky breadlike odour, became more prominent as the malting continued. The bags had also been observed to be slightly warmer than the room from 24 hours after the first soaking and beyond. After the soaking stages, roots emerged from the *Hordeum vulgare* seeds and began to attach to other seeds, requiring force to break them apart when stirring. G4 and especially G5 had shown small unidentified blue mould colonies on various seeds, but not in amounts that represented a safety concern. After dehydration, the groups further along in the malting process became less dense due to dried roots pushing the grains of malt apart. Grinding the malt also became significantly easier between groups, correlating with the extent the *Hordeum vulgare* grains were malted. After grinding, the differences between the scents of the groups became more prominent, with the earlier groups smelling like wheat flour, and the later, more malted groups (G4 and G5) smelling pungent, funky and sour. The more malted groups also showed more fine powders begin produced during grinding.

During the production of the liquid culture media, the early groups produced very thick, opaque and starchy media, while the more malted groups produced clear, brown-tinted solutions with fine sediment. The line between these states seems to occur at G3. After the media were used in the reactors and colonized, they all became clearer, and G2 formed channels that bubbles were observed to pass through. They all also formed an additional white layer above the sediment.

Figure 1: *The uncolonized control media samples in order of G1 to G5 from left to right*

Figure 2: *The used media samples from the reactors after 6 days of growth. In order of G1 to G5 from left to right*

During testing, the media from the reactors had a pungent, almost rancid smell with yeast, beer and bread notes. Within each group, the media looked visually identical, except for the control medium as shown above.

As the media was tested for specific gravity, the more malted media (G4, G5) flowed easily into the testing cylinder, while G3 was thick, and G1 and G2 were so thick that it was impossible to measure the specific gravities of the media, these qualities were shared with their control counterparts. After the wet-dry weight tests, some of the media samples visually turned into bread-like foams that crumpled when touched, while others formed hard cakes and fragments at the bottom of their crucibles. This behaviour did not have any pattern relating to the origin of the sample. During the pressure tests, remarkably few signs of respiration were noticed, aside from small bubbles that could plausibly have come from the stirring of the solution.

4. Analysis

4.1 Data Processing

Specific Gravity

The decrease in specific gravity is taken using the following formula, with the example of the first sample of the G3 group. Specific gravity is denoted as SP, and the decrease in specific gravity being ΔSP:

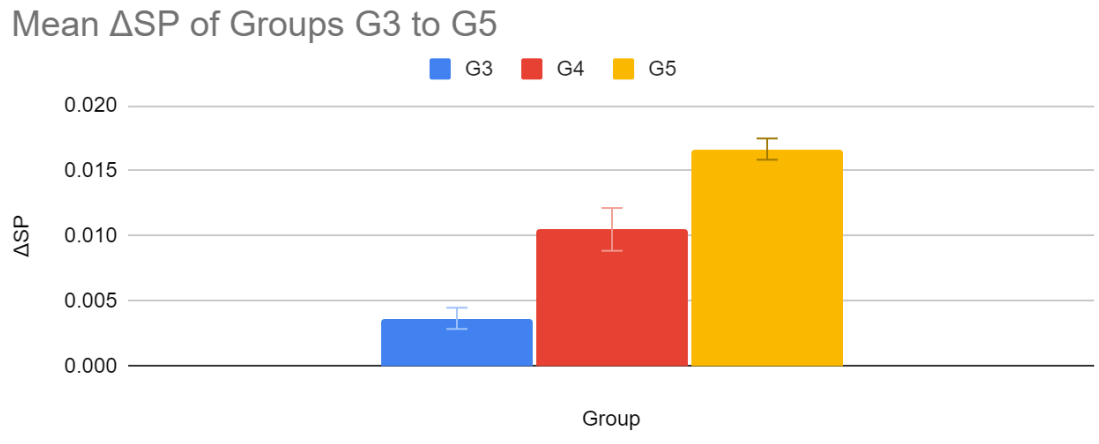
$$\Delta SP_{sample} = SP_{control} - SP_{sample}$$
$$\Delta SP_{first\ sample\ in\ G3} = SP_{control\ of\ G3} - SP_{first\ sample\ in\ G3}$$
$$\Delta SP_{first\ sample\ in\ G3} = 1.025 - 1.022$$
$$\Delta SP_{first\ sample\ in\ G3} = 0.003$$

This means that the specific gravity of the media decreased by 0.003 throughout its use in the first-sampled G3 reactor. Once the ΔSP is calculated for each sample, the mean of these values within the groups is calculated with the =AVERAGE function in Google Sheets, and the standard deviation is calculated with the =STDEV function in Google Sheets.

Table 5: *The ΔSP of the samples relative to the specific gravity of their corresponding pre-inoculation control media. The mean values and standard deviations of the groups are also shown. The identification numbers on the left-hand column are consistent with those on the raw data for this test, Table 1.*

	G3	G4	G5
1	0.003	0.011	0.018
2	0.004	0.012	0.016
3	0.005	0.009	0.017
4	0.003	0.008	0.017
5	0.004	0.012	0.016
6	0.003	0.011	0.016
Mean	0.004	0.011	0.017
Standard Deviation	0.000816	0.001643	0.000816

Graph 1: *A bar graph of the mean ΔSP of each group with error bars corresponding to the standard deviation of the group*



Viability

The increase in pressure (measured in kPa) during the viability test is calculated using the following formula, where P is the pressure and ΔP is the increase in pressure. An example is done with the G1 viability test results:

$$\Delta P = P_{final} - P_{initial}$$
$$\Delta P = 103kPa - 102kPa$$
$$\Delta P = 1kPa$$

This means that the pressure increased by 1kPa over the 200 seconds of the G1 viability test. This calculation is repeated for the other four groups, resulting in *Table 6.*

Table 6: ΔP (in kPa) during the viability test of groups G1 to G5

	G1	G2	G3	G4	G5
ΔP	1	1	0	0	0

The uncertainty of the pressure sensor is ±4kPa (and the uncertainty of ΔP is ±8kPa), meaning that due to uncertainty alone, these results do not indicate a significant change in pressure during the viability test. Therefore these results do not require further processing using graphs and statistical inference.

Wet-Dry Ratios

The wet-dry ratio, denoted as R, of the samples, is calculated using the following formula, with the example of the control sample of G1:

$$R = \frac{mass\ of\ dehydrated\ crucible - mass\ of\ empty\ crucible}{mass\ of\ wet\ crucible - mass\ of\ empty\ crucible} \times 100\%$$
$$R = \frac{17.762g - 16.974g}{27.443g - 16.974g} \times 100\%$$
$$R = 7.52698443\%$$

This results in a value that represents the percentage of non-evaporable mass in the samples.

The decrease in the wet-dry ratio, ΔR, is calculated by subtracting the R-value of a sample from the R-value of its control sample, shown below in the case of the first G1 sample:

$$\Delta R = R_{control} - R_{sample}$$
$$\Delta R = 7.52698443\% - 4.989793604\%$$
$$\Delta R = 2.537190826\%$$

This means that the proportion of dry matter in the liquid medium decreased by approximately 2.5% throughout the media's use in the first-sampled G1 reactor. These calculations are done for all samples, and the results are shown in *Table 7.*

Table 7: *The R and* ΔR values of each sample. *Note that the identification numbers on the left-hand side are consistent with the raw data for this test in Table 3.*

	R (%)	ΔR (%)
G1		
1	4.989793604	2.537190826
2	5.094546023	2.432438407
3	5.087592993	2.439391438
4	5.203150912	2.323833518
5	5.106480421	2.420504009
Control	7.526984430	
G2		
1	4.979004199	0.279019408
2	4.756980352	0.501043256
3	3.580463125	1.677560482
4	4.898305085	0.359718523
5	5.045871560	0.212152048
6	5.017215937	0.240807670
Control	5.258023607	

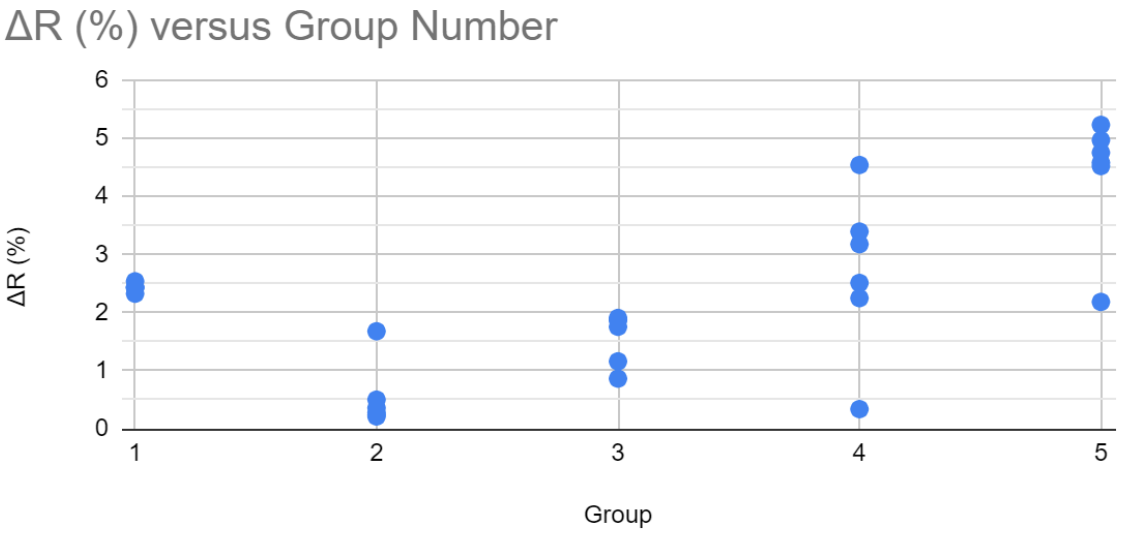
	R (%)	ΔR (%)
G3		
1	5.625817706	1.159651794
2	4.910918228	1.874551272
3	5.030849549	1.754619951
4	4.879182156	1.906287344
5	4.913494810	1.871974690
6	5.922165821	0.863303679
Control	6.785469500	
G4		
1	2.789598109	4.538168071
2	3.934904056	3.392862123
3	4.152397260	3.175368919
4	4.818583221	2.509182958
5	5.079962371	2.247803809
6	6.989443029	0.338323151
Control	7.327766180	

Table 7: *(Continued)*

G5		
1	3.798076923	5.228321033
2	6.844001108	2.182396848
3	4.061719991	4.964677965
4	4.276315789	4.750082167
5	4.448320977	4.578076979
6	4.507190459	4.519207497
Control	9.026397956	

The ΔR values may now be graphed in a scatter plot to visualize any correlation between the group and ΔR.

Graph 2: *a scatterplot with ΔR as the y-values and the corresponding group number as the x-value.*



4.2 Statistical Calculations

Statistical calculations will be performed on the processed data from the specific gravity and wet-dry ratio tests. In both cases, the correlation between the processed value and the group number (and hence the independent variable) is desired. Pearson's Correlation Coefficient is a suitable method for this task, as it produces a signed measure of the correlation between the variables and has an inferential counterpart to determine the significance of the result (Bargen). Pearson's Correlation Coefficient is calculated using the =PEARSON(y,x) formula in Google Sheets, y and x being the processed variable and the group number respectively. The test of statistical significance is performed using the table on https://www.biologyforlife.com/correlation.html and the absolute value of Pearson's r-value, the table will determine whether or not the p-value is over or under 0.05 or 0.01, but not what the p-value is exactly.

Using the ΔSP value from the specific gravity test and comparing it to the group number results in an r-value of 0.9802729376. This means that there is a strong, positive correlation between the decrease in specific gravity and the group number. To conduct the significance test, the null hypothesis, H_0, is that there is no significant correlation between the group number and the decrease in specific gravity. The alternative hypothesis, H_1, is that there is a significant correlation between the group number and the decrease in specific gravity. Using the aforementioned table, the r-value, and that the degrees of freedom is 16 (the number of pairs minus two), the p-value is determined to be less than 0.01. This means that the null hypothesis is rejected and that the strongly positive correlation is significant and unlikely to be caused by chance or sampling error. Therefore, the extent of *Hordeum vulgare* malting has a positive impact on the change (specifically decrease) in the specific gravity of *Saccharomyces cerevisiae* in a *Hordeum vulgare* malt-based liquid medium.

The same technique may be applied to the possible correlation between the decrease in the proportion of dry, non-evaporable matter in the reactors from the start of *Saccharomyces cerevisiae* growth to testing. Using the

group number as x, and the ΔR value as y, Pearson's Correlation Coefficient is determined to be 0.5953841421, a medium correlation between the variables. The null hypothesis, H_0, is that there is no significant correlation between the group number and ΔR. The alternative hypothesis, H_1, is that there is a significant correlation between the group number and ΔR. Using the aforementioned table, r-value, and that the degrees of freedom is 27 (29 pairs of xy values minus 2), the p-value is found to be less than 0.01. This means that the null hypothesis is rejected and that this medium positive correlation is significant and unlikely to be caused by chance or sampling error. Furthermore, a closer look at *Graph 2* shows that G1 does not follow the overall trend of the other groups and that excluding it from the calculations results in an r-value of 0.8439240795, a strong positive correlation, and a similarly low p-value, this will be discussed later in the exploration. Nonetheless, this result means that there is a medium correlation between the extent of *Hordeum vulgare* malting and the decrease of the proportion of dry matter in the *Hordeum vulgare* malt-based liquid medium after being used in *Saccharomyces cerevisiae* growth.

5. Evaluation

5.1 Conclusion

Overall, the hypothesis of this exploration is strongly supported. As the extent of *Hordeum vulgare* malting increased, both the specific gravity and proportion of the dry mass in the media samples decreased, both of these are indicators of *Saccharomyces cerevisiae* growth (Koutsokali and Valahas 2) (Hardwick) and strongly support the hypothesis. This is most likely to be caused by the increased breakdown of starch by α-amylase and ß-amylase during malting (Vinje et al.). A study by Vinje et al. also found that there were increased concentrations of nutrients used by *Saccharomyces cerevisiae* in malted *Hordeum vulgare* as the extent of malting increased, found by measuring their presence in the grains directly. This conversion of starch is also supported by the fact that the G1 and G2 media groups were extremely thick and starchy and only got thicker as they cooled during the liquid media creation portion of the procedure, this can be seen in the images of the media in *Figure 1* and *2*.This behaviour is very congruent with foods made with other starches, such as corn starch. This is also directly supported by the fact that in both tests the control values were directly related to the result of the test for that group. For example, the initial specific gravities were 1.025, 1.035 and 1.037 for G3, G4 and G5 respectively. The groups with higher initial specific gravities were also the groups that were observed to have the largest decreases in specific gravity.

One result that did not support the hypothesis was the result of the viability test. The results indicated that the yeast culture was either completely dead or had so few remaining cells that they could not be observed under the methods chosen. This could be due to a variety of factors, the most likely being the overconsumption of nutrients. The initial specific gravities of all media, the de-facto method of determining the growth potential of a wort in beer production, were significantly less than those seen in beer production, which also use yeast strains specifically developed for liquid culture conditions with reduced nutrients (Hardwick).

One of the most fascinating results of this exploration was the fact that the reduction of proportional dry mass in the G1 media was extremely consistent, not to mention at a similar level to those seen in G4. This could be caused by a variety of factors, including alternate metabolic pathways that are only possible in unmalted grain, such as those seen in bread baking. It could also be caused by something in the unmalted grain preventing ß-amylase from denaturing during media creation and sterilization, as ß-amylase is made *in planta* and activated by the embryo during germination (Vinje et al.). The ß-amylase could have then broken down starch molecules during the growth phase of the procedure along with those produced by *Saccharomyces cerevisiae.*

The results of this exploration support the use of the complete malting process of *Hordeum vulgare* in the creation of aerated liquid cultures for *Saccharomyces cerevisiae* for culture growth. More effective growth in cultures allows for faster and more efficient production of desired metabolites in natural and genetically modified *Saccharomyces cerevisiae* cultures for use in culinary, pharmaceutical and biotechnology industries.

5.2 Strengths

This exploration was carefully planned with an easily reproducible vertical process that allowed for the control of all procedural elements from the seed to the final product. All parts of the procedure were carefully planned such that almost any procedural or random error would equally affect all samples. The contamination

prevention practices were extremely sound with no contamination of any kind found. An appropriately sized trial with 29 samples was conducted, minimizing many sources of random error in testing and from unpredictable *Saccharomyces cerevisiae* growth. Moreover, two of the three quantitative tests had p-values of less than 0.01 and were conducted with equipment with very little uncertainty relative to the results obtained, leading to even more confident results. The standard deviation in the error bars in *Figure 2* did not come close to overlapping and increased confidence in the results of that analysis.

5.3 Limitations

There are only 5 batches of *Hordeum vulgare* malt and liquid media produced in the procedure, meaning a random error at any point during these portions of the experiment. For example, there could be a temperature variation in one liquid media batch due to the manual adjustments to the stovetop causing premature enzyme denaturing, or a hidden biological contamination in one bag of malt that caused differences in the enzyme catalysis of the malting process. A single random error in one batch could significantly affect a fifth of the data points used in the exploration. This source of random error can be remedied by extending the 5x6 nature of the reactors to the *Hordeum vulgare* malting and liquid media creation. This would also allow there to be more control data points to be available for additional analysis with statistical confidence.

A source of systematic error in the procedure is the viability testing procedure. While the other tests strongly supported evidence of *Saccharomyces cerevisiae* growth, this test did not. This could be caused by the small initial cell counts from the small media samples, the short amount of time given for testing the viability, and leakage in the testing apparatus. All of these errors would result in an artificially reduced or insignificant viability value from the test. This could be improved by using larger media samples to increase the initial *Saccharomyces cerevisiae* cell count, increasing the time the *Saccharomyces cerevisiae* cells have to perform aerobic respiration, and changing the testing apparatus to measure the volume of gas produced by the viability test culture instead of measuring a pressure change with a potentially leaky stopper. The final improvement would also eliminate the high amount of uncertainty of the Vernier Gas Pressure Sensor and hence increase any confidence in the results.

The results are hindered by the missing data points of the specific gravities of the G1 and G2 samples. They were unable to be measured due to their thickness and the testing apparatus available. Their values would have provided valuable context to the exploration and its results, such as whether or not the behaviour of the G1 group during the wet-dry ratio analysis was shared in both tests. This source of missing results could be resolved by using different, more resilient, testing equipment, or by diluting the media so that they can be measured and extracting the original specific gravities from that. Another method would have been to change the procedure such that all of the starch, the likely source of the thickness of G1 and G2, was filtered out of all of the media before use in the reactors.

5.4 Further Exploration

The results of this exploration open up numerous possibilities for further exploration. The behaviour displayed by G1 in the wet-dry ratio test is worthy of further research to conclude the source of the behaviour and its possible use as a time-saving method of a half-decent liquid culture media that does not require a complex malting process. Given the supposed positive trend of *Saccharomyces cerevisiae* growth with the extent of *Hordeum vulgare* malting, it would be interesting to see what happens when the malting process, specifically the germination phase, extends beyond the conventional procedure. Another interesting test would be to use a hemocytometer to measure the cell counts of *Saccharomyces cerevisiae* and compare it to the amount of respiration the culture is performing and if more metabolically productive cells are produced by different types of media. This idea can be extended to measuring the production of desired metabolites, such as ethanol or harvestable protein compared to the properties of the liquid media.

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Appendix:

Table A.3:

	Empty Crucible	Wet Crucible	Dry Crucible
G1			
1	7.781	12.19	8.001
2	9.803	*16.202*	*10.129*
3	11.015	15.182	11.227
4	*7.025*	11.849	7.276
5	*8.946*	13.313	9.169
Control	16.974	27.443	17.762
G2			
1	9.324	14.325	9.573
2	6.957	11.792	7.187
3	9.558	14.697	9.742
4	13.48	19.38	13.769
5	14.067	20.389	14.386
6	10.645	14.711	10.849
Control	17.826	28.077	18.365
G3			
1	15.128	22.007	15.515
2	6.862	11.24	7.077
3	6.944	11.158	7.156
4	7.874	12.178	8.084
5	7.146	11.481	7.359
6	7.729	13.048	8.044
Control	14.637	20.473	15.033
G4			
1	7.683	11.913	7.801
2	7.179	11.296	7.341
3	7.057	11.729	7.251
4	7.683	12.892	7.934
5	6.258	10.51	6.474
6	7.468	12.962	7.852
Control	7.186	11.976	7.537
G5			
1	6.934	11.094	7.092
2	9.742	13.351	9.989
3	*7.032*	11.439	7.211
4	9.091	14.563	9.325
5	9.873	16.752	10.179
6	13.431	19.133	13.688
Control	9.876	16.922	10.512