The role of molecular architecture in the hydration properties of brewers' spent grain

by
Jasmin S. Yang

A THESIS

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Abstract approved:_____

Michael H. Penner

An emerging strategy to reducing food waste is converting food processing byproducts to nutritious and inexpensive food ingredients. A relevant food processing waste stream is brewers' spent grain (BSG), the primary byproduct of beer production. BSG is a promising candidate as a food ingredient because of its high protein and fiber content, but it must be stored properly and dried sufficiently to prevent microbial spoilage. In this study, the effects of four storage conditions (Never Stored, Frozen (-20°C), Fridge (2°C), 16°C) and two drying conditions (HT: High Temperature, 65°C, 20% RH and LT: Low Temperature, 40°C, 50% RH) on the drying and hydration of BSG were analyzed through the determination of moisture sorption isotherms (MSIs). MSIs were determined by the climate box method and produced type-II sigmoidal curves. The MSIs for the Never-Stored BSG were similar for HT and LT drying, but for the three stored samples, LT-dried BSG absorbed more water in the a_w = 0.2-0.5 region. This implies that LT drying affected the capturing tendency of the BSG in terms of porosity and/or surface chemistry. These results provide insight on the hydration properties of oven-dried high-fiber food byproducts and discuss important experimental considerations of MSI determination.

Key Words: Brewers' spent grain, moisture sorption isotherm, rehydration, food waste

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I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Food Waste

The growing issue of food loss and food waste must be addressed for the development of sustainable food systems. The Food and Agricultural Organization of the United Nations (FAO) defines "food waste" as any food intended for human consumption that is "discarded, lost, degraded, or consumed by pests"[1]. This definition also includes food processing byproducts that are fed to animals or discarded in landfills[1]. Waste occurs along every step of the food supply chain (agricultural production, food processing, retail, and consumption). The FAO estimates that one third of global food production is lost or wasted, which has significant ramifications in all three pillars of sustainability: environmental, economic, and social[2]. Approximately 90% of wasted food is sent to landfills or incinerated which contributes to 8% of global GHG emissions (methane, carbon dioxide) and consequently, the intensifying climate crisis[3]. Other environmental concerns include the depletion of natural resources (soil nutrients, water, energy) and the impact of wasted food packaging materials[1,3]. From a business perspective, losses in marketable product pose a great challenge to food manufacturers; a 2010 study estimated that the United States lost $161.6 billion in 2010 due to food loss at the retail and consumer level alone[4]. For smallholder farmers in developing countries, a reduction of food waste has direct impacts on their livelihoods[2]. Waste disposal also comes with a high cost that places a burden on small businesses[1]. Food waste has significant implications in human health and social welfare due to food insecurity. An estimated 802 million people were food insecure in 2012, and this number will continue to increase due to the growing world population[4]. Although food insecurity is mainly a concern of income and purchasing power, the nutrient loss that occurs (mostly in the form of healthier foods, like fresh fruits and vegetables) is a relevant part of the conversation[3]. In developed countries, most food waste occurs at the consumer level, while in low-income countries, waste occurs earlier on in the supply chain in production or processing[1,2]. The challenge for industrialized countries lies in educating producers, retailers, and consumers of the problems with food waste and creating uses for wasted food that would otherwise end up in a landfill[2]. Source reduction must be considered in every step of the supply chain in order move towards more sustainable food production practices.

Waste Prevention Strategies

The United States Environmental Protection Agency (EPA) developed a Food Recovery Hierarchy that informs decisions in food production and waste management to best benefit the environment, society, and economy. The hierarchy is based on the EPA's general Waste Management Hierarchy and follows the concept of "reduce, recover, and recycle". The hierarchy suggests (in order of preference) source reduction, feeding hungry people, feeding animals, industrial uses, composting, and landfill/incineration as waste prevention strategies (Figure 1)[3,5].

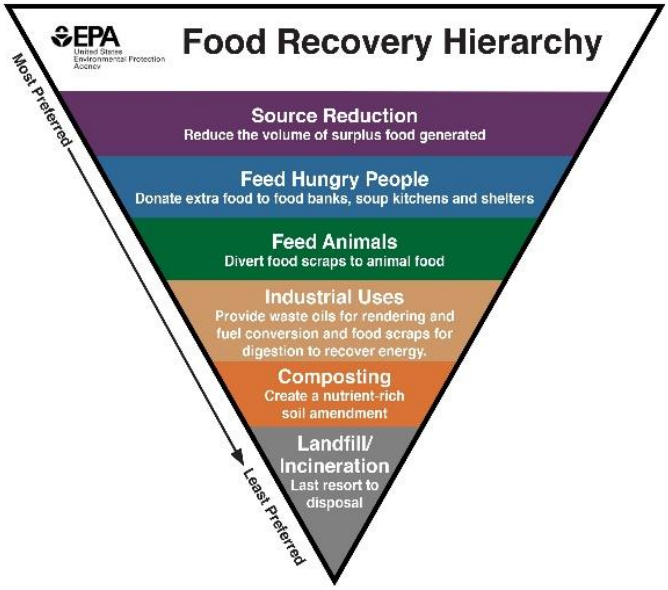

Figure 1. EPA Food Recovery Hierarchy[5].

From a food processor's standpoint, one strategy to reduce food waste and generate additional income is through the upcycling of food processing byproducts into food ingredients[6]. In many beverage products like wine, beer, and juice, primary source reduction is challenging or unavoidable. The suggested "source reduction" option of the Food Recovery Hierarchy can be achieved through repurposing food processing byproducts as ingredients in other foods. Byproducts from the food industry have the potential to be untapped sources of macronutrients, dietary fiber, and bioactive compounds; they can either be used in their whole form or processed to extract specific bioactive compounds[6]. Foods made with these byproducts can then be used to "feed hungry people", the next preferred option in the Food Recovery Hierarchy after source reduction. The utilization of food processing waste materials in novel food products has been proposed to provide nutrients to people living in the poorest regions of the world in which malnutrition and protein deficiency are a major concern[6]. This approach to source reduction of food waste has been explored in the context of many byproduct/product combinations. A challenge common to this approach is ensuring that the byproducts have the functional properties

necessary to be effectively used as ingredients in the targeted final food product and that overall consumer acceptance is not compromised.

Brewers' Spent Grain

A relevant food byproduct/processing waste stream is brewers' spent grain (BSG). BSG is the primary byproduct of beer production, comprising 85% of total byproducts obtained in the brewing process[7]. Every year approximately 39 million tons of BSG are generated globally, with the United States, China, Germany, and Brazil as the top producers[7,8]. At the start of beer production, malted barley is milled, mixed with water, and heated to enzymatically degrade the starch into fermentable sugars. This stage, known as mashing, produces a sweet liquid (wort) that is later fermented into beer. Traditionally, the wort is filtered through the insoluble residue of the grain in a lauter tun, but some larger breweries are now using mash filters that allow for better extraction of wort[8]. In both cases, the insoluble residue left after filtration is known as brewers' spent grain (Figure 2A)[7]. BSG consists primarily of the seed coat-pericarp-husk layers of the barley grain and other insoluble cell wall materials (Figure 2B)[7]. Although the specific composition of BSG differs based on the barley variety, brewing conditions, and other ingredients that may be added, BSG is high in fiber (approximately 50% on a dry weight basis) and protein (up to 30%), making it a promising candidate for value-added food applications[8]. The fiber is made up of cellulose, hemicellulose (largely arabinoxylans), and lignin (polyphenolic plant cell wall component). Minor elements of BSG include lipids and ash that make up 10% and 2-5% of the product, respectively[8].

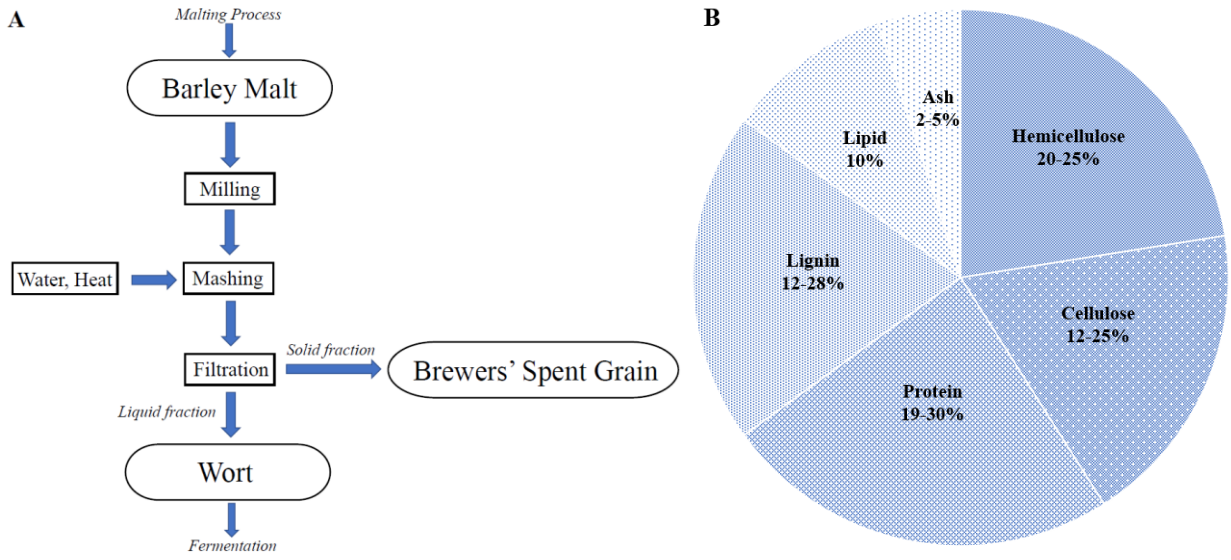

Figure 2. (A): Flow chart of BSG production in the brewing process (adapted from Mussatto et al.[7]). (B): Typical composition of BSG produced from barley (adapted from Lynch et al.[8]).

Current practices in the waste management of BSG in breweries include use as animal feed, composting, or disposal in a landfill. Even if BSG is sent to farms as feed, there is often an oversupply for larger breweries[9]. Because of the high moisture content of BSG (77-81% (w/w)) and fermentable sugar content (residual after filtration), BSG is prone to rapid spoilage due to microbial activity[7]. The sheer weight and volume of the material also makes transportation costly and cold storage difficult. Dehydration of the spent grain is the preferred processing method to reduce weight and prolong the time it can be stored before it spoils. BSG has been dehydrated using various methods including freeze drying, oven drying, and superheated steam drying, but currently, the only feasible option for most breweries is oven drying due to the lack of specialty equipment required for other drying methods and the high cost to operate them[8].

A means of converting BSG to useful food ingredients through dehydration would (1) alleviate costly waste handling fees for brewers and (2) create an affordable source of supplemental fiber and protein that can be used as ingredients in foods. BSG as a food processing byproduct lends itself to value-added processing, as it is already in compliance with all food safety regulations in the brewery[10]. To utilize BSG in this capacity, the development of economically viable technologies for fiber preparation (storage, drying, milling, etc.) is necessary to ensure sensory and nutritional properties are not diminished. It is essential to understand the hydration properties of dried BSG because the incorporation of fiber ingredients into food largely depends on rehydration. Hydration properties are influenced by what we are defining as “molecular architecture”, which includes porosity, surface chemistry, and bulk density. This work will focus on relating experimental results of hydration to these microstructure elements. Answering fundamental questions on the chemical basis of BSG hydration may be advantageous for the applications of the following experimental findings to other high-fiber food processing byproducts.

Food Preservation via Dehydration

Dehydration is one of the oldest and most common forms of food preservation[11]. While once limited to sun-drying, humans have developed many more methods for dehydration that have allowed for the development of many shelf-stable food products. One limitation that comes with

dehydration, however, is that rehydrated foods are often of inferior quality to the pre-dried product. The main factors affecting the rehydration of foods are porosity and the presence of capillaries and cavities near the surface of the dry material[11]. Structural changes that can occur during drying including shrinkage and the potential collapse of pores, which would impede rehydration. One notable drying method is freeze drying which produces very porous products with high rehydration rates. Freeze drying requires long processing times and extensive energy expenditures, making it a very expensive option for commercial use[11]. This prompted our interest in investigating thermal methods of drying BSG. The motivation for understanding the molecular nature of dehydration/rehydration processes is to improve processing technologies for a variety of high-fiber food processing byproducts, ultimately to reduce food waste.

CHAPTER 2. THEORY OF REHYDRATION.

Water Activity

For centuries, people have been preserving food by salting, sugaring, freezing, and drying foods: all which contribute to the reduction of water activity[12]. Now we understand that water activity (a_w) describes the amount of water in a food that is available to act as a solvent, undergo chemical reactions, and support microbial growth, which makes it arguably the most important property of water in a food system[13,14]. A food with an a_w below 0.6 is considered a dehydrated food, and in most cases, will not support microbial growth (bacteria, yeasts, and molds)[12]. Because of this, a_w is an important consideration in food safety/processing and is often used as a critical limit in food safety plans for food processing facilities. Some of the reactions that are affected by a_w include nonenzymatic and enzymatic browning, lipid oxidation, protein denaturation, starch gelatinization, and starch retrogradation[14]. Water activity must be carefully controlled in foods to maintain quality and prolong shelf life.

Water activity is a thermodynamic description for water. Water activity is defined as the ratio between the equilibrium vapor pressure of water in a food (p_f) to the vapor pressure of pure water (p_w) at the same temperature[12]:

$$a_w = \left(\frac{p_f}{p_w}\right)_T \tag{1}$$

While vapor pressure is often used in food science to calculate a_w, the original equation is related to fugacity, or the "escaping tendency of a substance"[15]. The composition of a food and the conditions in which measurements are made affect the apparent a_w of a food. While a_w does not fluctuate much with temperature changes in high-moisture foods, it can increase with an increase in temperature for low-moisture foods[14].

In this study, the instrument used to determine a_w uses the dew-point method of measurement. In this method, a sample is allowed to equilibrate with the air in the headspace of a sealed chamber ($p_f = p_{air}$). Once the sample is equilibrated in the chamber, the temperature of a mirrored surface in the chamber is lowered until the mirror fogs, indicating the air has reached its saturation vapor pressure (dew-point temperature). The condensation is detected by an optical sensor and the temperature measured by a thermocouple. The measured dew-point temperature is used to determine the a_w of the sample (this relationship is well-characterized by psychrometric charts)[14]. The dew-point method is convenient and fast, but one disadvantage is that it only accounts for surface a_w[13].

Moisture Sorption Isotherms

Moisture sorption isotherms (MSIs) are widely used to characterize the hydration properties of food products. MSIs relate a food's water activity to its moisture content. This information is important in the food industry because it influences decisions in food processing, storage, and even packaging[16]. Practically, MSIs can be used to determine critical water activities in foods (e.g. a_w at which a crispy food becomes soft), and predict which chemical reactions may occur[15].

It is generally recognized that there are five types of MSI curves; types I, II, and III are the most relevant for foods (Figure 3)[15].

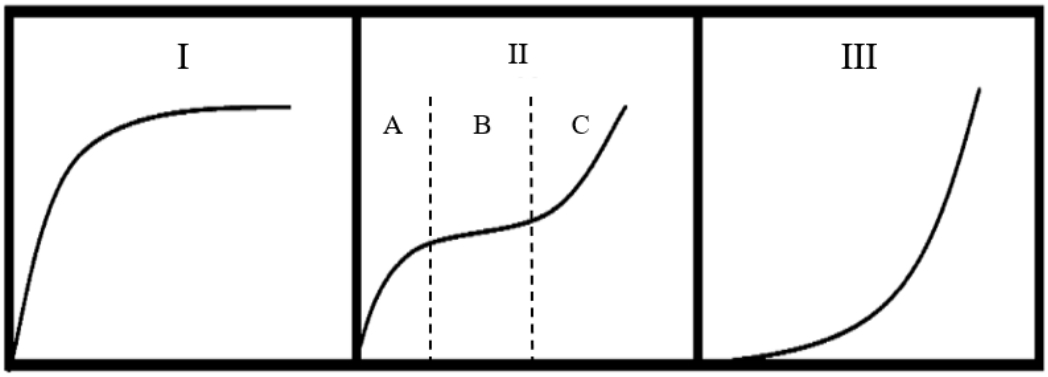

Figure 3. Characteristic curves of type I, II, and III MSIs. Reproduced from Barbosa-Cánovas (2007) with modifications[15]. The x-axis is a_w, and the y-axis is mositure content. Regions in the type II isotherm include: A) monolayer, B) multilayer, and C) condensed capillaries[17].

Type I isotherms are usually observed for foods that can absorb large amounts of water at low water activities (e.g. anticaking agents). Once this initial absorption occurs, a complete monolayer is achieved and moisture content does not change much even with an increase in RH[15]. Type II isotherms describe most foods and their more complex shape is reflective of the interaction of capillary effects, solid surface-water interactions, and effects of Raoult's law on its water absorption[15]. The sigmoidal curves of type II isotherms have "bending regions" in the lower (a_w = 0.1-0.2; Figure 3A) and upper ranges (a_w > 0.5; Figure 3C) in which larger changes in moisture are required to change the relative humidity of the food[15]. Three distinct regions have been defined in type II isotherms as the monolayer, multilayer, and condensed water regions (A, B, and C, respectively in Figure 3). In the monolayer (Figure 3A), each hydrophilic functional group in the food is associated with a water molecule(s); water molecules are tightly bound and therefore unavailable to mobilize solutes and allow reactions to occur[12,15]. Mathematical models including the Guggenheim-Anderson-DeBoer (GAB) equation have been used to estimate the solid surface area of the sample at the monolayer stage[17]. The middle region (Figure 3B) is a transition phase between the two in which multilayers of water fill the capillaries of a food[17]. The last region of Type II isotherms (Figure 3C) describes "condensed", "bulk", or "free" water that has similar mobility as pure water[15]. The term "condensed" refers to the condensation of the water molecules in capillaries when the saturation vapor pressure of water is achieved; the condensed form of water then dictates the a_w of the sample[18]. Type III isotherms are observed for crystalline foods (e.g. sugars, salts), as moisture gain is minimal until enough water is present to dissolve the crystal[15].

The shape of moisture sorption isotherms reveals a lot about a material's ability to rehydrate, which is important for dried foods like brewers' spent grain when considering its storage or use as an ingredient. In type II isotherms specifically, the slopes of the curves in each of the monolayer, multilayer, and free water regions provide information on the "relative water capturing tendency" of the food at different moisture contents and relative humidities. Because the vapor pressure of the saturated salt solution is constant (i.e. the collision frequency between

water molecules and the surface of the sample is the same), some regions of the food must "capture" water better than others (presumably polar regions and within pores). Thinking in terms of "capturing tendency" is the inverse way of thinking in terms of the "escaping tendency" of water, which relates to the original definition of water activity as a relative fugacity.

Methods for Determining MSIs

The simplest method of determining moisture sorption isotherms is the "climate box method". Although it is not as sensitive compared to newer methods (pressure plate method, sorption microbalance method, sorption microcalorimetric method), results using the climate box method still provide relatively accurate results[19]. In this method, samples are stored in airtight boxes alongside saturated salt solutions of known relative humidities. During this storage period, the a_w of the samples will slowly equilibrate with the saturated salt solutions due to the migration of water vapor from the solutions into the sample or vice versa. Table 1 shows the relative humidity values (25°C) of nine saturated salt solutions commonly used for MSI determination because of their wide range of RH values[20]. Equilibration can take weeks to months to achieve depending on the size of the sample, and may require the placement of small vials of toluene in the climate box to prevent mold growth during this time, particularly for the higher a_w salt solutions[17,19]. Temperature should be held constant among all climate boxes, as the relative humidities of saturated salt solutions are temperature dependent. Once the samples have reached equilibrium, moisture content is determined gravimetrically.

Solution	RH at 25°C
LiCl	11.30 ± 0.27
CH_3COOK	22.51 ± 0.32
MgCl	32.78 ± 0.16
K_2CO_3	43.16 ± 0.39
$Mg(NO_3)_2$	52.89 ± 0.22
NaCl	75.29 ± 0.12
KCl	84.34 ± 0.26
KNO_3	93.58 ± 0.55
K_2SO_4	97.30 ± 0.45

Table 1. Water activities of saturated salt solutions used in moisture sorption isotherm determination (adapted from Greenspan, 1976)[20].

CHAPTER 3. APPLICATIONS OF BSG IN FOODS.

The incorporation of BSG has been studied in many different food systems including bread, pasta, snacks, and even frankfurters[21,22]. BSG contains dietary fibers, proteins (with essential amino acids), minerals, polyphenols, vitamins, and lipids, making it a healthful addition to existing food formulations[21]. The primary non-cellulosic polysaccharides in BSG are arabinoxylans, which are known to have cholesterol-lowering properties, fecal bulking effects, and also the ability to enhance the absorption of certain minerals[7,23]. The addition of 30% BSG in bread formulations can increase the dietary fiber content up to fivefold[10]. Despite these health benefits, there are challenges in using BSG as a food ingredient due to its flavor, color, and texture; consumer studies show that foods containing BSG are only acceptable if BSG is incorporated in small quantities (5-10%)[7].

Most applications of BSG are in bread products which require drying and milling of the BSG into flour[8]. In general, the addition of dietary fiber to breads is known to have a negative impact on quality parameters[10]. High-fiber flours tend to absorb more water than typical wheat flours, which decreases the availability of water for the hydration of starch and gluten in bread-making. This also affects starch gelatinization and hinders the development of a strong starch-gluten network, contributing to lower loaf volumes[24–26]. Dietary fiber also affects mixing properties (increase of dough development time and dough stability) and fermentation, which make adjustments in processing necessary[10]. Some studies have shown that the addition of enzymes (e.g. amylases, pentosanases, lipases, etc.) to doughs containing BSG can improve loaf volume, texture, and even increase shelf life[25].

An additional consideration is the pre-processing (drying, milling) of BSG before it is used in food formulations. Prentice & D'Appolonia (1977) analyzed the effects of drying temperatures on the final bread quality and consumer acceptance. They found that heating dried BSG at high temperatures (150°C) causes the final bread product to be less desirable by consumers overall[27]. This is in agreement with Mussatto et al. (2006) that suggest BSG should be dried below 60°C to prevent the development of off-flavors[7]. This low drying temperature reflects BSG flour recipes

posted online, in which various organizations including the American Homebrewers Association, Brewers Association, and Brooklyn Brew Shop recommend drying BSG in the oven at 150-200°F (66-93°C) for 6-7 hours[28–30]. These organizations also advocate for the use of BSG in brewpub kitchens or for baking at home, which shows that the incorporation of BSG into food items is possible and acceptable. The Brewers Association echoes that the use of 5-10% of BSG in baked goods will not be noticed by consumers[29].

Water Holding Capacity of BSG

To our knowledge, there have not been published studies that analyze the hydration properties of BSG from a theoretical standpoint of microstructure and porosity, but there have been several reports on the water absorption capacity (WAC) and water holding capacity (WHC) of BSG flours and how they affect rheological properties of bread doughs[26,31]. WAC is measured by mixing a sample with excess water and measuring the volume of the hydrated sample with a graduated cylinder; it is expressed as the volume of the hydrated sample divided by the sample weight (mL/g)[32]. WHC is measured by mixing a sample with excess water for a given time, then centrifuging and draining the supernatant. The pellet is weighed, then dried to determine its dry weight. WHC is expressed as the mass of the water that is retained by 1 g of sample (i.e. mass of retained water divided by mass of dry sample)[33]. WHC specifically is of interest when considering the nutritional benefits of dietary fibers in general, but not all fibers improve colonic health with the same efficiency. The relative strength of water binding to dietary fibers largely affects stool weight, which makes WHC and the understanding of moisture sorption isotherms important for foods intended to improve colonic function[17]. Aprodu et al. (2017) found that the WAC and WHC of BSG were very similar to those of commercial fibers (oat, pea, wheat), and Ktenioudaki et al. reported slightly lower values[26,31]. Variation is not surprising as the exact chemical nature of BSG changes depending on the malt and processes used in that specific brew. While many of the physiochemical properties of BSG have been explored, they have not been explained in the context of microstructure and particle size.

CHAPTER 4. HYDRATION PROPERTIES OF BSG

Study 1: Drying and equilibration curves of BSG

Materials and Methods

BSG Preparation and Storage. Fresh brewers' spent grain from a lager-style malt mash was collected from the Pilot Brewery at Oregon State University (Figure 4A). Following sample collection, BSG was either dried immediately or stored in plastic bags at -20°C (Frozen), +2°C (Fridge), or 16°C for three days (Figure 4B).

BSG Drying and Milling. BSG was spread in a thin, uniform layer on metal sheet pans (Figure 4C) and dried in a forced-air dryer (MP-2000, Enviro-Pak Division of Tech-Mark, Inc., Clackamas, OR, USA) under two conditions: HT (High Temperature, 65°C; 20% RH), and LT (Low Temperature, 40°C; 50% RH). Small samples of BSG were collected every 30 min for HT drying and every 60 min for LT drying to track changes in a_w. Water activity (measured at 25.0 ± 0.4°C, AquaLab PRE Water Activity Meter, Decagon Devices, Inc., Pullman, WA) was determined after allowing samples to cool to room temperature in sealed containers. Drying ended when the a_w of the collected sub-sample was less than 0.5 ($a_w < 0.5$ was sufficient to prevent microbial spoilage during storage). The longest drying time was overnight (~16 h); a_w values ranged from 0.413 to 0.672 for the dried samples. Dried BSG was milled into a fine powder using a knife mill fitted with a 2.00 mm mesh (SM100, Retsch GmbH, Germany).

Figure 4. BSG sample preparation. (A): Fresh BSG collected from the lauter tun; (B): BSG stored in a temperature-controlled room; (C): BSG spread on trays before drying in the oven.

Water activity equilibration between BSG and saturated salt solutions in sealed containers. Initial experiments were done to estimate the time required for the a_w of oven-dried BSG samples to equilibrate with that of selected saturated salt solutions in sealed containers (desiccators). This type of information was deemed necessary for the experimental design of

subsequent MSI experiments. In the first experiment, a ~1 g sample of milled BSG (Frozen, LT drying) was placed in an open-topped plastic water activity measurement container (cylindrical cups purchased from the manufacturer of water activity measurement instrument, AQUALAB; ~3.89 cm diameter, 1.14 cm height, ~5 mm sample thickness) and stored in a sealed desiccator alongside an uncovered beaker of saturated LiCl solution (a_w = 0.113) at room temperature. Water activity measurements were made throughout the equilibration time, with the of the sample in the same desiccator after the measurement was taken (91 h total). A second experiment was done to compare water activity equilibration times for HT and LT oven-dried samples stored at analogous relative humidities. In this experiment, ~1 g samples of milled BSG (Never Stored, HT and LT drying) were first "pre-dried" to low a_w using the method described above with saturated LiCl for 2 days. The term "pre-dried", as used here, refers to the removal of water and consequent lowering of water activity from already oven-dried BSG samples. This step was necessary to lower the water activity of the samples for subsequent adsorption isotherm experiments. Following the pre-drying, samples (one each of LT and HT dried BSG) were transferred to desiccators containing uncovered beakers of either saturated K_2CO_3 (a_w = 0.432) or KCl (a_w = 0.859) and stored at room temperature. All saturated salt solutions were prepared by dissolving the salt in DI water in excess (visible precipitate). Water activity measurements were made periodically throughout equilibration (166 h total).

Results

Time courses for oven-drying of BSG. Typically, drying curves are determined using the moisture content rather than water activity, but we chose to use water activity due to its convenience and relevance to BSG spoilage. The propensity of microbial spoilage is largely determined by water activity, making it a suitable parameter for the purposes of this study. Visually, there was no perceptible difference in appearance or color of any of the samples after drying (HT versus LT, Figure 5).

Figure 5. Samples of BSG (before milling) in each storage condition before and after drying in LT conditions.

The LT conditions (lower temperature, higher %RH) and HT conditions (higher temperature, lower %RH) were chosen to demonstrate two extremes of drying. Never stored BSG samples were well below the target water activity (a_w = < 0.031, target a_w = 0.5) within 3 h of drying in HT conditions. In contrast, the water activity of the same starting material only reached 0.406 after 13.5 h in the LT condition. However, this LT drying trial result most certainly represents a minimum rate of drying for the specified type of dried because the dryer fan settings were incorrect (lower air circulation than anticipated), which likely increased the drying time required to achieve the target a_w. For HT drying with the stored samples, the curve for the 16°C stored BSG most resembled that of the Never Stored sample. Target a_w values were reached after 240 min, 180 min, and 180 min for the Frozen (a_w = 0.087), Fridge (a_w = 0.075), and 16°C (a_w = 0.338) stored BSG respectively. For the LT samples, even after 450 min of drying, the Frozen and Fridge stored BSG was still not under a_w = 0.5.

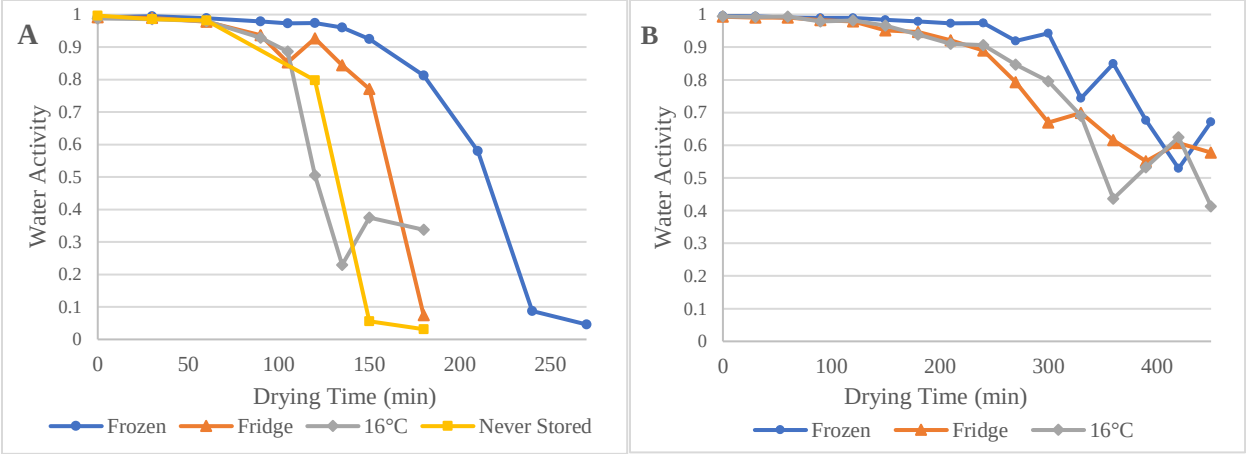

Figure 6. Drying curves of BSG at (A) HT: 65°C, 20% RH and (B) LT: 40°C, 50% RH for each storage condition (3 days storage). Note: no data was available for the Never Stored BSG at LT conditions so it is not depicted in part B of this figure.

Desorption equilibration experiments using a Frozen/LT oven-dried BSG sample having a starting a_w of 0.727 and a saturated LiCl solution as the target a_w demonstrate the extended periods of time required for samples to truly achieve equilibrium. The data presented in Figure 7 show that even after 91 h of storage, the milled BSG only reached a_w = 0.137, which is slightly above the expected a_w = 0.113 (Figure 7). Adsorption equilibration experiments showed that similar, relatively long periods of time were required for samples to reach equilibrium. Pre-dried samples (a_w approx. 0.15) stored in the desiccator with the saturated K_2CO_3 solution reached an a_w of 0.428 after almost 2 days of storage, which is very close to the expected a_w of the solution (0.432). The a_w of this sample was maintained at approximately 0.430 for 5 additional days with only minor fluctuations, which may have been caused by slight temperature changes. For samples in the desiccator with the saturated KCl solution (a_w = 0.859), the a_w values of all samples were lower than expected (HT a_w = 0.799, LT a_w = 0.792) even after 166 h (~7 days) of drying (Figure 8).

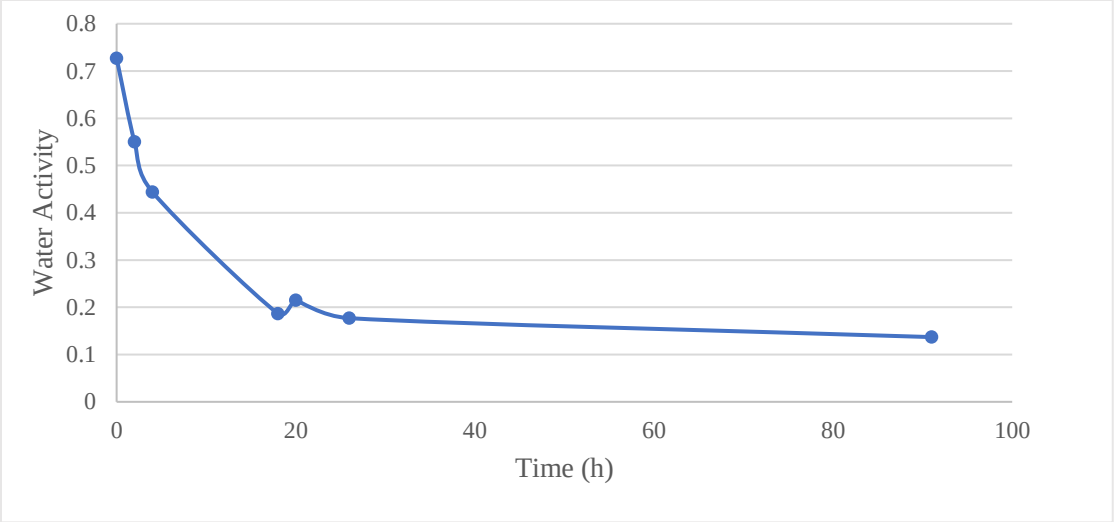

Figure 7. Time course of changes in water activity for BSG samples (Frozen 3 days, LT drying) stored in a sealed desiccator alongside saturated LiCl solution (a_w = 0.113) at room temperature.

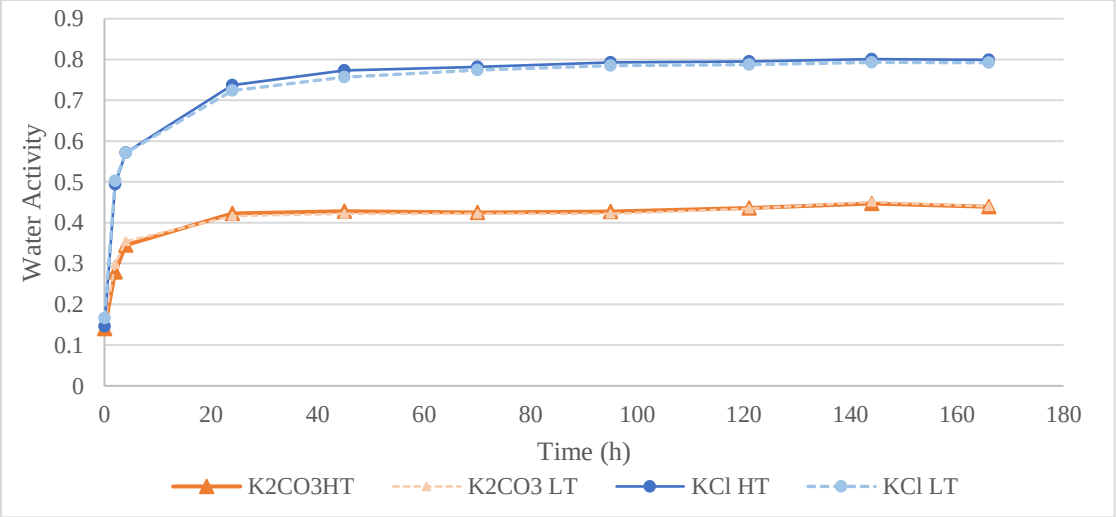

Figure 8. Time course of changes in water activity for pre-dried BSG samples (Never Stored, HT (solid lines), LT (dashed lines)) stored in sealed desiccators alongside saturated salt solutions: K_2CO_3 (a_w = 0.432; triangles) and KCl (a_w = 0.859; circles).

Discussion

Drying Curves. The purpose of this study was to estimate the time it takes to dry BSG to a_w < 0.5, and to observe differences in drying kinetics among the BSGs stored under four plausible conditions. This information was to be used as an aid in the design of approaches for sample preparation for subsequent moisture sorption isotherm studies. It is widely accepted that

microbial proliferation does not occur below $a_w = 0.6$[12], so a value of 0.5 was chosen because it is well below that threshold and provides a buffer in the case of moisture absorption from the atmosphere during storage. For HT drying, the Fridge and Frozen samples took longer to dry than the Never Stored and 16°C BSG (Figure 6A). This can be explained by the fact that the Frozen and Fridge samples were at a lower starting temperature than the other two samples and required more time to heat before evaporation could begin; in fact, the Frozen sample was only partially thawed when put in the drying oven and some BSG remained in frozen clumps. The water activity of the Frozen sample did not change in the first 140 min of drying, which is likely because the ice in the same was melting. In the case of LT drying (Figure 6B), the a_w fluctuated greatly, even increasing at some time points. Variability is expected in both drying conditions due to the non-homogenous nature of BSG and sampling from different parts of the tray. Because the LT dried BSG decreased in a_w at a slower rate (i.e. smaller decrease of a_w from one time point to the next), the variability is much more apparent. The undried samples in Figure 4 show the different anatomical fractions of BSG, with the husk layer and fragments of the starchy endosperm being the most visually apparent[8]. Visual examination of the samples during the drying cycle suggested the starchy and husk components dried at different rates, therefore the ratio of husk to starch in a sample is likely a large determinant of measured water activity. In future drying kinetics studies, multiple samples should be taken to assure representative sampling; separate experiments could be done in which the anatomical fractions are separated and the drying kinetics of each fraction determined independently.

Equilibration with Saturated LiCl. Early on in the equilibration of samples with LiCl (could be thought of as a secondary drying; referred to as pre-drying), a_w measurements show that the initial drying happens very quickly, then slows. It is interesting to note the slight increase of water activity from t = 18 h to t = 20 h; this suggests that once the BSG dries to a sufficiently low a_w, then even the act of removing it from the desiccator and measuring the a_w could allow detectable adsorption of moisture from the atmosphere. Based on the results from these experiments, a pre-drying time of 3 days was chosen for future MSI experiments.

Equilibration with Saturated K_2CO_3 and KCl. There was no appreciable difference between the adsorption rates (as measured by a_w) of the LT and HT dried samples (Figure 8). It is important to note that the similarities in adsorption could be an artifact resulting from the fact that both

samples were pre-dried via equilibration with the LiCl solution after having already been dried in the oven. This secondary drying (pre-drying) may obscure the differences in the sorption properties of the BSGs if non-reversible changes in microstructure occurred. The samples stored with the saturated KCl solution seemed to plateau in their extent of hydration well before equilibrating with the salt solution. One consideration would be the incomplete saturation of the KCl solution. This explanation is not plausible, however, as lower concentrations of the salt would lead to a_w values of over 0.859. Perhaps simply more time was needed to reach this equilibrium, which is a common issue for this method of MSI measurement. Basu et al. (2006) also note that in the climate box method (as used in these experiments), it is difficult to maintain high relative humidity environments[34]. Choi et al. (2001) suggest that there is decreased surface diffusion of the water after achieving full monolayer coverage, which might explain that equilibrium for samples with K_2CO_3 solution (a_w = 0.432, which approximates the a_w for monolayer/multilayer coverage) was reached much quicker than with KCl (a_w = 0.859, which approximates the a_w for relatively dilute aqueous solutions)[35]. In addition, the dew-point method of measuring water activity only measures surface equilibrium relative humidity, so this may also be a contributing factor[13]. In a moisture sorption isotherm study of food fibers by Cadden (1988), samples of an unspecified mass were allowed 4-6 weeks to equilibrate with the salt solutions[17]. Another study by Yan et al. (2008) stored dried banana samples with saturated salt solutions until constant weight (15-30 days), but had larger samples than this study (5 g)[36]. Ertugay and Certel (2000) only equilibrated cereal samples with saturated salt solutions for 5 days[16]. Variation in equilibration times is expected because of different sample sizes and densities. One reason for the extended equilibration time reported by Cadden in particular, was that water activities were not measured after equilibration; moisture contents of those samples were related to the theoretical a_w of the saturated salt solution, making complete equilibration crucial for MSI determination. Although equilibrium may not be reached, we chose a storage time of 5 days and measured the true a_w of the samples immediately before preparing the samples for moisture determination. Even if equilibrium is not achieved, the true a_w of the sample at that moisture content would be reflected through this measurement.

The results from Study 1 provided a basis for the BSG preparation methods used in Study 2, which focuses on the moisture sorption isotherms of representative BSG preparations. It also

suggests that changes in BSG microstructure that result from drying may impact rates of equilibration with saturated salt solutions.

Study 2: Moisture Sorption Isotherms of Milled BSG

Materials & Methods

Milled BSG Sample Preparation. Fresh brewers' spent grain from a lager-style malt mash was collected from the Pilot Brewery at Oregon State University. Storage and drying conditions were identical to those used in Study 1. Frozen samples were thawed slightly before spreading on the sheets to dry; this allowed for the distribution of BSG in a thin layer (rather than large pieces). HT samples were dried for 3 hours, and LT samples were dried for 16 hours to $a_w < 0.5$. Halfway through drying, the BSG was stirred. The a_w of the dried BSG was measured after cooling to room temperature in a sealed container. Dried BSG was milled to pass a 2 mm screen (i.e. relatively fine powder) as described in Study 1. Milled BSG samples were stored in air-tight glass jars at room temperature until being used.

Moisture Adsorption Isotherms. To determine the moisture adsorption isotherm of dried and milled BSG, the climate box method was used. Approximately 1 g samples were pre-dried in a desiccator with saturated LiCl solution ($a_w = 0.113$) for 3 days. Samples were then transferred into desiccators alongside open containers of saturated salt solutions that yielded varying relative humidities (Table 1). Samples were stored at RT for 4-5 days. Saturated salt solutions were prepared by dissolving salts in DI water in excess (visible precipitate). All salts used in the MSI analysis were reagent grade.

Water activity measurements of each BSG sample was taken immediately after removing the sample from the specified desiccator. Immediately after measuring water activity, the same sample was transferred to an aluminum moisture determination cup and weighed to the nearest 0.1 mg for subsequent gravimetric moisture determination. Moisture content was determined gravimetrically according to standard procedures adapted from the AOAC Official Method 925.09 (vacuum drying at 70°C, 29 mmHg, 16 h)[37].

Results

Initial Conditions of Dried BSG. The initial water activities of all BSG samples were under the target (a_w < 0.5) after forced-air oven drying in both conditions. Compared to Study 1, the a_w values of the BSG after drying were much more consistent among the storage and drying conditions (Study 1 final a_w values ranged from 0.0315 to 0.672, while Study 2 final a_w values ranged from 0.211 to 0.381). This could be because the samples in Study 2 experienced continuous drying without changes in heat and humidity from periodic sampling, as well as more uniform spreading of the Frozen BSG sample on the sheet pans by allowing it to thaw before drying. There could also have been impacts of oven position, as there were multiple trays dried at the same time (2 trays for Never Stored, 6 trays for Stored). For samples of all storage conditions, the HT samples had a lower a_w than the LT samples. After milling, the a_w of all samples (except the Frozen-HT sample) increased slightly due to absorbance of moisture from the atmosphere, but all remained under the a_w < 0.5 target value.

	Whole BSG		Milled BSG	
Sample ID	a_w	Temp (°C)	a_w	Temp (°C)
Never Stored-HT	0.3275*	25.2	0.332	25.3
Never Stored-LT	0.34*	25.3	0.405	24.9
Frozen-HT	0.296	25.3	0.277	25
Frozen-LT	0.381	25.2	0.387	24.9
Fridge-HT	0.211	25.3	0.245	25
Fridge-LT	0.351	25.2	0.363	24.8
16°C-HT	0.25	25.3	0.289	25
16°C-LT	0.347	25.2	0.359	24.9

Table 2. Water activities of whole and milled HT and LT oven-dried BSG samples used for MSI determination. HT samples were dried for 3 h and LT samples were dried for 16 h. *These values are expressed as the average of a_w values measured from two trays.

Equilibration. Pre-dried samples (stored with LiCl, 3 days) were equilibrated undisturbed for 4-5 days in desiccators with saturated salt solutions. Although some samples reached equilibrium with the saturated salt solutions they were stored with (LiCl, CH_3COOK, MgCl, K_2CO_3), equilibrium was never achieved for higher a_w solutions, particularly KCl, KNO_3, and $K(SO_4)_2$ (a_w values are given in Figure 9). The highest a_w achieved with $K(SO_4)_2$ was 0.878 for the Never

Stored samples, which is ~0.1 units less than the expected a_w of 0.979 at room temperature. The greater the RH of the saturated solution, the greater the deviation between the actual a_w of the BSG samples and the theoretical a_w for the saturated salt solutions they were incubated with (Table 1). Samples stored in desiccators with either KNO_3 or $K(SO_4)_2$ for the complete incubation period exhibited powder caking as a result of water absorption.

Moisture Sorption Isotherms. The moisture adsorption isotherms of all samples appeared to follow a type-II sigmoidal curve, which is characteristic for most foods that behave like non-porous or macroporous adsorbents[38]. In Type II MSIs, the beginning of the linear middle section (around $a_w = 0.2$) is understood to indicate complete monolayer coverage and the start of multilayer adsorption[38]. Interestingly, in the MSIs of stored BSG samples, there appears to be a difference in this region of the curve between the HT and LT isotherms for the three stored samples (Figure 9; comparing the blue versus orange lines within each plot). The LT samples seem to require a higher moisture content to achieve complete monolayer coverage compared to the HT BSG samples. In all three stored samples, the LT and HT curves seem to overlap once $a_w > 0.5$ and hence, their final moisture contents are very similar. In contrast, the Never Stored sample appears to have been impacted by the oven-drying conditions (LT versus HT) in a different region of the MSI. In the higher water activity regions ($a_w > 0.8$) of the Never Stored sample curves, the moisture content (MC) of the samples dried under LT conditions are significantly higher than the analogous samples dried under HT conditions. It is not clear whether or not these differences are significant, as only one replicate of each treatment was done, but trend established by the higher MC/a_w data points certainly argues that it is significant. There are several possible explanations for the differences in water absorption in the different BSG samples that will be explored below.

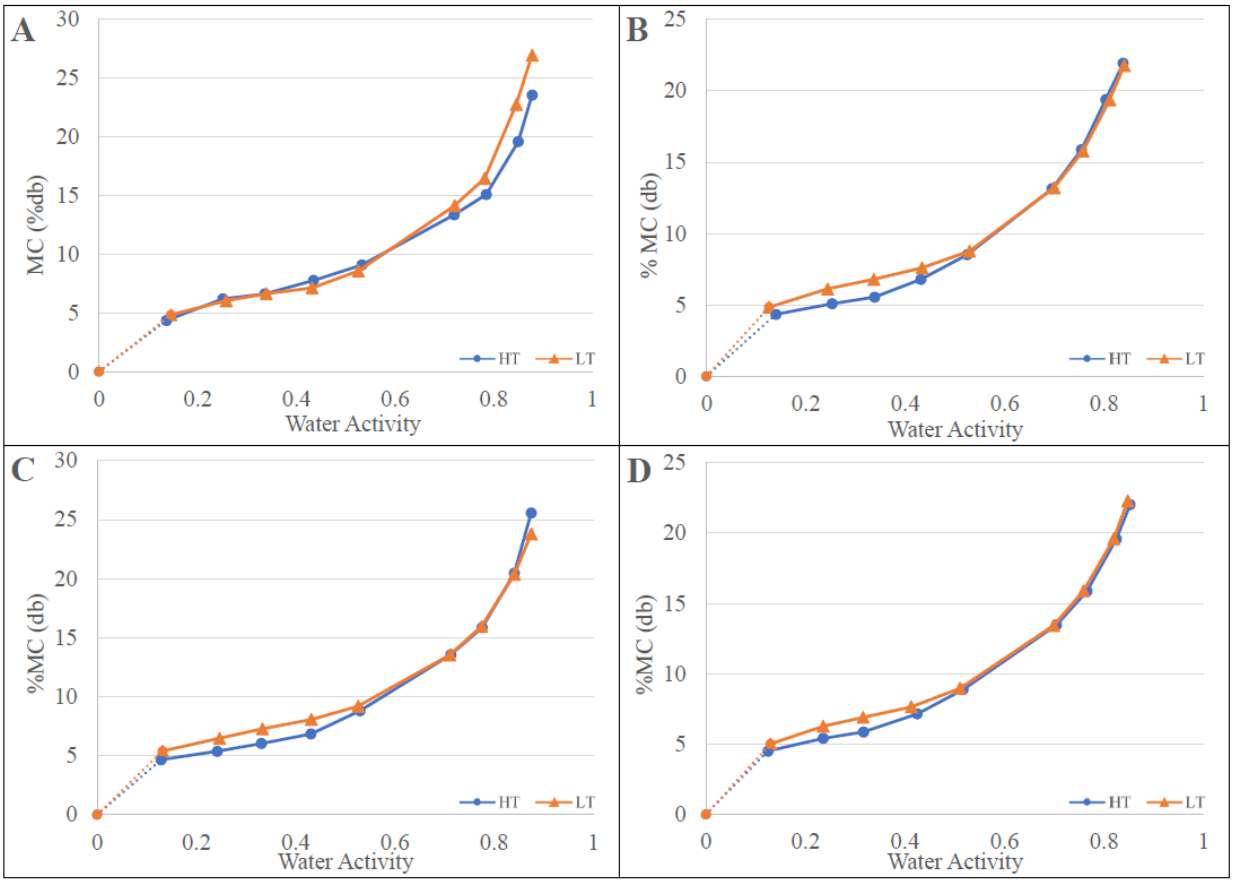

Figure 9. Moisture sorption isotherms of HT (65°C, 20% RH, 3 h) and LT (40°C, 50% RH, 16 h) dried/milled BSG stored under different conditions: (A) Never Stored, (B) Frozen, 3 days, (C) Fridge, 3 days, and (D) 16°C, 3 days. Moisture contents are expressed on a dry basis. Dotted lines trace back to the 0% MC, $a_w = 0$ point (curve shape unknown). Data points represent single measurements for each experimental condition.

Discussion

Initial Conditions of Dried BSG. One relationship that was observed in both Study 1 and Study 2 is that for a BSG sample at a given moisture content, the measured water activity is positively correlated with temperature. When considering the relative humidities of HT and LT drying (20% and 50% respectively), the lowest possible water activity for BSG dried in these conditions is 0.2 when measured at 65°C for HT conditions, and 0.5 when measured at 40°C for LT conditions. In Study 1, HT-dried BSG samples were at water activities far below 0.2, and in Study 2, LT-dried BSG samples were at a_w values far below 0.5 when measured at 25°C. This signifies that the a_w of the sample decreases as the temperature of measurement decreases. This

relationship is important for manufacturers to consider if they are drying BSG to a specific water activity.

Equilibrium. Results from Study 1 showed that the BSG samples stored with higher a_w salt solutions did not reach the expected a_w values in the allotted time. The same phenomenon occurred in Study 2. As previously discussed, it is not likely that the a_w values for the saturated salt solutions themselves were off, or that temperature fluctuations caused this discrepancy. In the case that equilibrium was reached between the BSG samples and saturated salt solutions in the chambers, one possible explanation for the low a_w is that during the transfer of the sample from the chamber to the water activity meter, the loosely-bound water on the surface of the sample evaporated. Care was taken to transfer the sample from the desiccator to the water activity measurement instrument as quickly as possible, but the results from Figure 7 (in Study 1) show that the act of removing the BSG sample from the desiccator can cause fluctuations in a_w. Authoritative texts indicate that a limitation of the dew-point method of water activity measurement is that it only accounts for surface a_w[13]. We interpret this to mean that equilibrium is only achieved between the air in the measurement chamber and the surface of the sample (it is unclear what is defined as "surface"). Because surface water molecules would most readily evaporate, the surface would be slightly drier than the rest of the sample; this may explain why the measured a_w was lower than the theoretical a_w of the saturated salt solution. An alternative consideration is that BSG samples may never have reached equilibrium with the saturated salt solutions during the storage period. Because the adsorption of water vapor starts from the surface of the BSG flour (~ 5 mm sample bed in the water activity sample cup) and diffuses inward, an unequilibrated sample would exhibit a higher surface a_w than overall a_w. This increased adsorption on the surface of the BSG flour sample is supported by previous observations of caking in the BSG incubated with the two highest a_w saturated salt solutions. "Humidity caking" occurs when water is absorbed from the environment and makes surface particles "sticky". This allows for the formation of liquid bridges that drive particle attraction, while the interior parts of the powdered sample bed are still dry[39]. Unfortunately, incomplete equilibration and the caking phenomenon would both cause the a_w of the sample to be higher than the theoretical value, which is not what we observed.

More information is necessary to determine why the a_w of the samples did not align with theoretical values. With modified climate boxes that allow for the weighing of BSG samples, the equilibration of the sample with the saturated salt solutions could be ensured by incubation until constant weight. Future experiments to determine the effect of humidity caking on the measured a_w could include incremental incubation of BSG samples with saturated salt solutions of increasing relative humidities (effectively slowing water adsorption to allow time for water molecules to diffuse to the interior of the sample bed), or by measuring a_w before and after mixing a caked sample. In the case that samples were not truly equilibrated, the limitation of surface a_w measurements when using the dew point method of water activity measurement must be factored into the interpretation of the resulting MSIs.

Effects of Drying Conditions on MSIs. Porosity is one of the main drivers of rehydration. Pore structure (e.g. pore volume, pore density) defines surface area, and therefore impacts the opportunities for hydrophilic binding and capillary condensation of water[17]. Typically, drying food at a lower temperature (specifically below the glass transition temperature, T_g) results in the maintenance of pore structure[40]. Higher temperatures may cause shrinkage of the entire product and the collapse of pores as well[40]. Our experimental results suggest that at a given relative humidity, more moisture has been absorbed by the LT dried BSG than the HT dried samples for the Frozen, Fridge, and 16°C stored samples. This means that the LT samples had a higher "capturing tendency" of water vapor during the equilibration with the saturated salt solutions for a_w values ranging from 0.2 to 0.5. This increased absorption is consistent with the preservation of porosity with drying at lower temperatures. When considering the results in Figure 9 from the perspective of monolayer formation, the linear portion of the LT samples start at a higher MC than for the HT samples for the stored samples. This suggests that the LT samples had a higher surface area per unit weight, therefore requiring more water molecules to form a monolayer. There are mathematical models that can determine the linear portion of the MSI in a more rigorous way but are beyond the scope of this study.

It is important to note that porosity may not be the only factor that accounts for the difference in water absorption. Xiong et al. (1991) argue that rather than porosity, the composition and surface chemistry of samples is the defining factor of absorption. Their findings on desorption isotherms of extruded pasta suggest that monolayer adsorption is driven by the polar groups that exist

throughout the sample, which is independent of pore size[41]. This could be an explanation for the similarity in the LT and HT dried MSIs for the Never Stored samples in the low and intermediate a_w regions. LT dried BSG had increased water adsorption in different regions of the MSI depending on the storage conditions, specifically the high a_w region (> 0.8) for the Never Stored sample and intermediate a_w region (0.2-0.5) for the stored samples. The trend observed in the Never Stored sample appears to be significant because all four points of the LT curve are higher than those of the HT curve. If this trend is real, the molecular rationale for this behavior is still unclear at this point. This leads to the conclusion that the differences between LT and HT dried stored samples could be a result of changes that occurred in storage (see comments on microbial growth in the following section).

Effects of Storage Conditions on MSIs. Figure 9 shows that all three storage conditions (Frozen, Fridge, and 16°C) yielded very similar MSIs in terms of the observation that a higher moisture content is required for complete monolayer coverage of the LT samples. Having not done chemical analyses of the BSG samples, we can only infer changes in BSG composition due to microbial and endogenous enzyme activites[42], but these are not likely to occur to any great extent under some of the storage conditions (i.e. Frozen and Fridge). Robertson et al. (2010) found that after five days of storage at 20°C, microbial populations increased 1000-fold[42]. Due to microbial activity, there was a noticeable decrease in total sugar and starch content for samples stored at both 4°C and 20°C, but no compositional changes were observed in the frozen (-20°C) sample[42]. Faulds et al. (2008) reports that endogenous carbohydrases and proteases can be active during BSG storage[43]. Another group demonstrated that endogenous enzymes can solubilize 2% of the total carbohydrates in BSG to glucose and trace amounts of other sugars[44]. Solubilization was determined by suspending BSG in 50 mM ammonium acetate buffer at pH 5 for 5 h at 50°C[44]; the pH is similar to that of the BSG used in this study (pH = 5.4), and 50°C is even higher than the LT drying temperature (40°C) , signifying that enzymes may have been active during drying. Marousis et al. (1989) suggests that the presence of sugars or soluble carbohydrates decreases the porosity of dried starch due to precipitation of sugars[45]. The presence of these sugars or oligosaccharides could effectively “clog” the pores during drying. If this is the case, the samples with the highest sugar content (Never Stored and Frozen, based on minimal microbial activity) should have the least accessible pores and therefore, lower overall absorption and a lower monolayer MC. In the case of the frozen sample, freeze-thawing could also have effects on

porosity. As water expands upon freezing, existing pores in the BSG may have been enlarged, contributing to increased porosity after drying (assuming that cell structure was not destroyed when the ice melts). These larger pores could offset the effects of the sugar still present in the sample and therefore allows the Frozen sample to absorb water with similar efficiency as the samples with lower sugar (Fridge, 16°C).

Milling. The collapse of pores during the milling process is intuitive. Milling or grinding reduces BSG particle diameter and may also effect other physiochemical properties, including surface chemistry and hydrophilicity[46]. Cadden found that in the case of a highly processed fiber (oat bran), particle size reduction did not affect physiochemical properties (i.e. density, water holding capacity). For wheat bran, however, the grinding effect of the roller mill caused its matrix structure to collapse. In beer production, malted barley is first milled then mashed—both of which likely caused significant damage on the fiber matrix even before post-drying milling. Although the mill used in this study was a knife mill (less damaging than a roller mill), we assume that very little of the original cellular structure of the barely is maintained. Milling also increases the surface area of fibers, which could be a contributing factor to water absorption[46]. Particle size distribution is also an important consideration and can be determined using a sieve analysis. Measurements of bulk density and particle density would also be of interest, as both of these parameters are related to porosity[47].

It is also important to note that all the samples underwent a secondary drying phase during the pre-equilibration of the samples in the desiccator containing LiCl. This was necessary to obtain a moisture adsorption isotherm (starting from low MC) but may have had effects on the porosity of samples (e.g. collapse of pores). Secondary drying with saturated salt solutions has been used by other groups, and is a better alternative to oven drying (a harsher drying method)[36]. This type of drying is a relatively slow process that occurs at room temperature. Changes in sample properties due to this treatment per se are not known. The important thing to keep in mind is that this type of drying was the final step for all the samples prior to the actual MSI experiments. Therefore, differences in the hydration properties of the various samples prior to the pre-drying step could be somewhat obscured by this treatment.

CHAPTER 5. CONCLUSION & FUTURE INVESTIGATION

The results presented in this study provided insight into the nature of experimental methods aimed at understanding the hydration properties of potential byproduct ingredients in foods and the hydration properties of brewers' spent grain in particular. The results were interpreted based on well-accepted molecular models used to explain the adsorption/absorption of water by foodstuffs; in particular, surface hydrophilicity/hydrophobicity and porosity/surface area.

Through drying experiments, we observed a positive correlation between water activity and temperature for samples at a given moisture content. All the MSIs followed the sigmoidal shape of the classic type II isotherm. The MSI results indicate that for the stored BSG dried under LT conditions, more water was required to achieve full monolayer coverage in the a_w range from 0.2 to 0.5. This suggests that storing BSG and drying it at a lower temperature can allow for increased water adsorption at a given relative humidity; some explanations include the preservation of porosity in LT drying, and the decrease of soluble solids (sugars) in the stored samples due to microbial activity. Results also indicate that for the Never Stored BSG dried under LT conditions, more water was adsorbed in the sample at high a_w regions ($a_w > 0.8$) in condensed capillaries. It is difficult to make conclusions regarding the effects of porosity on the observed increase in water adsorption in these regions without explicit measurement of pore sizes and densities.

In a more general sense, results from both Study 1 and 2 provide information about experimental methods used in MSI determination, particularly with the dew point method. Incomplete equilibration with saturated salt solutions as well as humidity caking can be sources of error that carry through to the interpretation of MSIs. If the removal of low a_w or high a_w samples from desiccators for only a short time can cause a significant change in the measured sample a_w, it is vital that precautions are taken to prevent vapor exchange with the atmosphere in the widely used climate box method of MSI determination. In terms of water adsorption, MSI results suggest that storage and drying conditions can have an impact on the hydration properties of fibrous foods.

There are still questions that have yet to be answered regarding other elements of microstructure and molecular architecture. The priority is to perform more replicates of the MSI experiments to

confirm the accuracy of the results presented in this thesis. Temperature control is something that must be considered in the future, as MSIs are sensitive to temperature changes[16]. It would also be of interest to see the extent to which the parameter effects observed for BSG are applicable to other byproduct/fiber ingredients.

Future studies should include actual measurements of surface chemistry parameters (hydrophilicity/hydrophobicity) and porosity so that correlations can be documented. It would also be informative to see how other functional and physiochemical properties relate to the water adsorption characteristics of these samples. This includes measurements of water absorption capacity, water holding capacity, bulk density, particle density, and particle sizes of the BSG samples. Visualizing the pores of the LT and HT samples through scanning electron microscopy would also allow estimates of pore size. The characterization of pores as micropores (< 2 nm diameter), mesopores (2-50 nm diameter), and macropores (>50 nm diameter) is important because it defines adsorption behavior (e.g. dominance of surface forces or capillary forces)[35]. If the BSG samples are macroporous, then pore size is likely insignificant in relation to surface chemistry; in such cases surface adsorption is likely the dominant factor dictating the differences in absorbance observed through the MSIs.

With the final goal being the incorporation of BSG into different food products as a high-fiber ingredient, it is also important to compare how LT and HT dried BSG responds in food systems and whether the postulated increased porosity of LT dried samples is significant in the processing and quality of the final product. In addition, due to the variation among the BSG from different brews, it would be interesting to see if the trends observed in this study also apply in other cases. An energy usage analysis also needs to be conducted to understand whether LT drying for such a long time (16 h versus 3 h for HT drying) is worth the potential increase in water adsorption at low water activity ranges in the dried BSG. This would be important information for breweries that need to consider the upfront cost of industrial drying equipment, cost of operating the dryer, as well as the energy and time expenditure necessary for BSG processing.

Repurposing brewers' spent grain as a food ingredient is an environmentally and socially responsible alternative to using it as animal feed or compost. It is important to understand the hydration properties of BSG to inform decisions in storage, processing, and product formulation.

By applying the basic theory of rehydration, capillary action, and porosity, this information may be applied to other high-fiber food processing byproducts that have the potential to be food ingredients as well.

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