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WASTE VALORIZATION IN THE ALCOHOL BEVERAGE INDUSTRY

Applications for co-product streams of beer breweries and whisky distilleries

Thesis
CENTRIA UNIVERSITY OF APPLIED SCIENCES
Environmental Chemistry and Technology
September 2020

ABSTRACT

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Degree programme Environmental Chemistry and Technology		
Name of thesis WASTE VALORIZATION IN THE ALCOHOL BEVERAGE INDUSTRY. Applications for co-product streams of beer breweries and whisky distilleries.		
Instructor Egidija Rainosalo		**Pages** 35
Supervisor Jana Holm		

The production processes of whisky distilleries and beer breweries are explained and compared. The resulting waste streams are considered regarding their chemical and biological properties. A review is done on current and possible conversion pathways for these waste streams to create value added products.

Extraction methods concerning the valorization of spent hops are discussed and compared, and an overview of possible valorization pathways is provided. New challenges concerning some of these waste streams due to recent market developments are described.

Previous literature on the topic is reviewed and recent findings are presented. Case studies on the technological processes are presented for each of the co-product streams. These include spent hops, spent yeast, spent grain and pot ale. It is found that research is ongoing in all these fields and that the understanding thereof is rapidly evolving. Finally, an overview is provided of the current waste streams and their respective possible value-adding applications.

Key words
Alcoholic Beverage Industry, beer, brewery, distillery, spent grain, spent hops, spent yeast, spent trub, valorisation, value added products, waste, whisky

CONCEPT DEFINITIONS

ABI – Alcoholic Beverage Industry
Apoptosis – a form of programmed cell death
Autolysis – Breakdown of yeast cells due to the yeast’s own enzymes
BOD – Biological Oxygen Demand
BSG – Brewers Spent Grain
BSY – Brewers Spent Yeast
COD – Chemical Oxygen Demand
Draff – A liquid similar to pot ale but more dilute
Eukaryote – Organism in which the cell has a nucleus within a nuclear envelope
IPA – India Pale Ale, a beer style well known for its strong hop additions
LED – Light Emitting Diode
PBR – Photobioreactor
Pot Ale – A liquid or Syrup that remains after alcohol has been removed to the distillation process
Spent Trub – A mixture of proteins, hops and other debris resulting from the brewing process

ABSTRACT
CONCEPT DEFINITIONS
CONTENTS

1 INTRODUCTION

Significant amounts of solid, liquid and gaseous waste are disposed in the alcoholic beverage industry. The repurposing of such waste to convert it into a co-product with added commercial value is called valorization. Both whisky and beer are made from cereal and the production process is somewhat similar, therefore it follows that the generated waste side streams may also be similar in composition. Accordingly, it could be assumed that valorizing the waste streams of these two different industries utilizes similar methods, which may be modified depending on the type and properties of the given material.

This review compares the production process of both beverages and examines the currently available literature for novel valorization possibilities. Which waste streams are generated in the production of beer and whisky? What is the chemical composition of these waste streams, and how could they be valorized? To acquire value added products from waste streams, extraction technologies are often used. Which extraction processes are used specifically? When evaluating these, emphasis will lay on extraction methods that do not require supercritical solvents.

One of these waste streams, spent hops, apparently has not received as much attention as other waste streams, therefore a focus is laid on comparing extraction possibilities for this stream. Furthermore, due to the strong similarities between the spent hops and hemp industry waste, extraction methods for both are considered and the cross application thereof, between the two industries, is suggested.

2 OVERVIEW OF BEER PRODUCTION

To understand the side- and waste streams that are generated during the brewing process, it is imperative to understand how beer is produced. Due to the abundance of literature on this topic, for example in the works of Narziß or Stewart, it is herein only described in a way that is necessary for such comprehension (Narziß & Gastl, 2017; Anstruther, Stewart, Russell, 2017). Beer is made from cereals that have been malted. The malting and kilning process itself is complex and again there are abundant resources on this topic, such as the works of Briggs (Briggs D.E., 1998).

During the production of malt from grain, an incomplete natural germination process is exploited, which will cause a series of enzyme degradation of the cereal endosperm. Furthermore, due to this process, soluble protein and enzyme activity is increased, while starch is broken down into simple sugars. The degradation of polysaccharides into fermentable carbohydrates is an essential aspect of malting (Gallaghar, Gupta, Abu-Ghannman., 2010, 319). To produce beer, the malted grain is milled to increase the solubility of its constituents. Chemical and enzymatic conversions are also aided by milling the malt (Narziß & Back, 2009, 185). Milled malt is called grist, which is transferred into the mash ton, where the mashing process takes place. Here the grist is soaked in 63-74°C warm water, causing the degradation of starch which in turn directly leads to maltose and dextrin. This is mostly done by α- and β-amylase (Narziß & Back, 2009, 238).

The water which now contains the dissolved malt constituents is called wort. As the grains are not further necessary for beer production, they are removed in a process called lautering, which is mainly a physical separation process (Narziß & Back, 2009, 397). This process results in Brewers Spent Grain, which will henceforth be abbreviated as BSG. When the wort has been separated from the solids, it is concentrated by boiling. Furthermore, boiling destroys the malt enzymes, stabilizes the wort, decreases the amount of coagulatable nitrogen, partially dissolves hop constituents and removes some of the more undesirable malt and hop aromas (Narziß & Back, 2009, 503).

Shortly before the end of boil, hops are added to isomerize their compounds and impart bitter flavor to the beer. Polyphenols contained within the hops assist the coagulation of proteins and hop essential oils are supposed to impart hop aroma in some beer styles (Narziß & Back, 2009, 544). The hops do not remain in the beer in most cases, but are removed before fermentation, resulting in spent trub. The finished wort is chilled somewhere between 5-25°C while being transferred to a fermentation vessel and finally being inoculated with yeast. During fermentation, the yeast will convert sugars into mainly alcohol and carbon dioxide (Narziß & Gastl, 2017, 231).

After fermentation has taken place the yeast is removed to avoid autolysis and the negative effects thereof, leaving the brewer so called 'green beer', which must mature before it can be finally bottled and sold. Brewers spent yeast is a direct result of fermentation. The inoculation of wort with yeast is a process that requires diligent sterilization, and it is described in Chacon’s work alongside the beer fermentation process (Chacon, 2020). The brewing process and its waste streams have been graphically displayed in Figure 1 for easier understanding. In summary, the brewing process generates the following side streams: BSG, BSY, spent hops, carbon dioxide and expired beer if it is not sold in time.

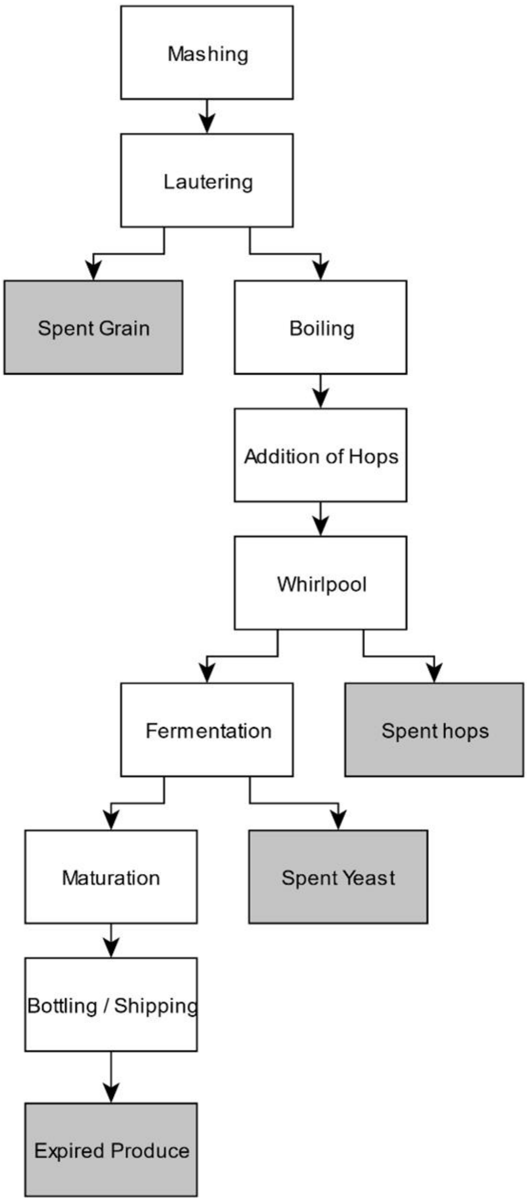

FIGURE 1: The beer brewing process and some of the waste streams it generates

In Germany, a country that is well known for its beer industry, there were 1341 breweries in the whole country in 2012. Of these 632 are situated in Bavaria. However, around 49% of all breweries produce less than 1000 hL of beer, providing only 0.2% of the domestic production (Gaida, Schüttmann, Zorn & Mahro. 2013). While small breweries have not been a recent development in Finland, the current shift of customer preference towards small breweries is quite noticeable (Silovsky, 2016). By knowing the amount of waste that is produced per hectoliter of beer, the total amount of annually generated co-products could be estimated for a country or a new brewing enterprise. Using this information, it is possible to design a waste management strategy that is tailored towards the business. The following data shows the approximate amount of waste produced by a brewery.

It should be noted that these are not precise measurements, as the amount of waste produced can differ significantly within the brewery depending on the style of beer being brewed as "heavy" styles like stout require a different malt bill than "lighter" styles like sessions ales. This can easily be proven by observing different beer recipes, of which there are many available online. TABLE 1 was created by using the data from Mathias, Mello & Sérvulo, who have adapted it from 3M (Mathias, Alexandre, Cammarota, Mello, Sérvulo, 2015).

TABLE 1: Amount of brewery waste streams produced per hL (adapted from Mathias et al., 2015).

WASTE STREAM	**AMOUNT [KG] PER hL OF BEER**
Spent Grain	14-20
Hot Trub (including hops)	0,2 – 0,4
Residual Yeast	1,5 – 3
Diatomaceous Earth	0,1 – 0,2

It follows from the data shown in TABLE 1 that the most voluminous "solid" waste streams are BSG and BSY, however it should be kept in mind that higher volume does not automatically translate into higher value. Therefore, creating high value products from spent trub may be viable considering the possible pharmaceutical and chemical applications of it, but it would require extensive research. The main waste streams in the brewery industry have been identified by Mathias et. al. and the corresponding information is shown in TABLE 2. While diatomaceous earth slurry is considerable waste stream in bigger breweries, it is not always used in large quantities in small breweries. Therefore, it is not considered in this work.

TABLE 2: Main components in the brewery wastes (adapted from Mathias et al, 2015).

PARAMETER	**SPENT GRAIN**	**HOT TRUB**	**RESIDUAL YEAST**	**DIATOMACEOUS EARTH SLURRY**
Fibers	Yes	-	-	-
Carbohydrates	-	Yes	Yes	-
Protein	Yes	Yes	Yes	Yes
Free amino acids	Yes	-	Yes	-
Ash	Yes	Yes	Yes	-
Vitamins	Yes	-	Yes	-
Phenolic Compounds	Yes	Yes	-	Yes
Fatty Acids	-	Yes	Yes	-
Fossil Materials	-	-	-	Yes

3 PRODUCTION OF WHISKY

While the production of whisky is in parts like the brewing of beer, there are some key differences. Firstly, whisky is a distilled spirit while beer is not. Secondly, there are usually no hops added to the wort when whisky is made. The source material for whisky can be either pure malt or unmalted grains, depending on the type of whisky produced. Even though grain distilleries rely on unmalted grains as a base material for all further endeavors, they still require some of the enzymes that malted grain provides and therefore add a small fraction of it to their unmalted grain (Anstruther, Stewart, Russell, 2017, 75). The grain undergoes the mashing and lautering process, leaving the distiller with wort. Unlike brewers, distillers do not boil the wort (Anstruther, Stewart, Russell, 2017, 144).

The fermented wort is then subjected to distillation, in which the more volatile compounds are separated from the less volatile ones, which remain as pot ale. During subsequent distillation, spent lees remain. There are different types of stills, but for the sake of readability they will not be further discussed in this work, as they all rely on similar thermochemical principles. It may be noted however, that a reflux still may allow for a finer distinction between the waste streams as it allows to remove unwanted compounds more effectively than a pot still. During distillation, the head and the tails are separated as well, these are volatile compounds that are not desired in the finished product. The initial distillate may be distilled again several times. (Anstruther, Stewart, Russell, 2017, 170). Afterwards the spirit is matured in wooden barrels, where it undergoes some chemical changes over time. In Figure 2, the distillation process is shown schematically. The production of whisky mainly generates spent grain, spent lees, spent yeast and pot ale as co-products.

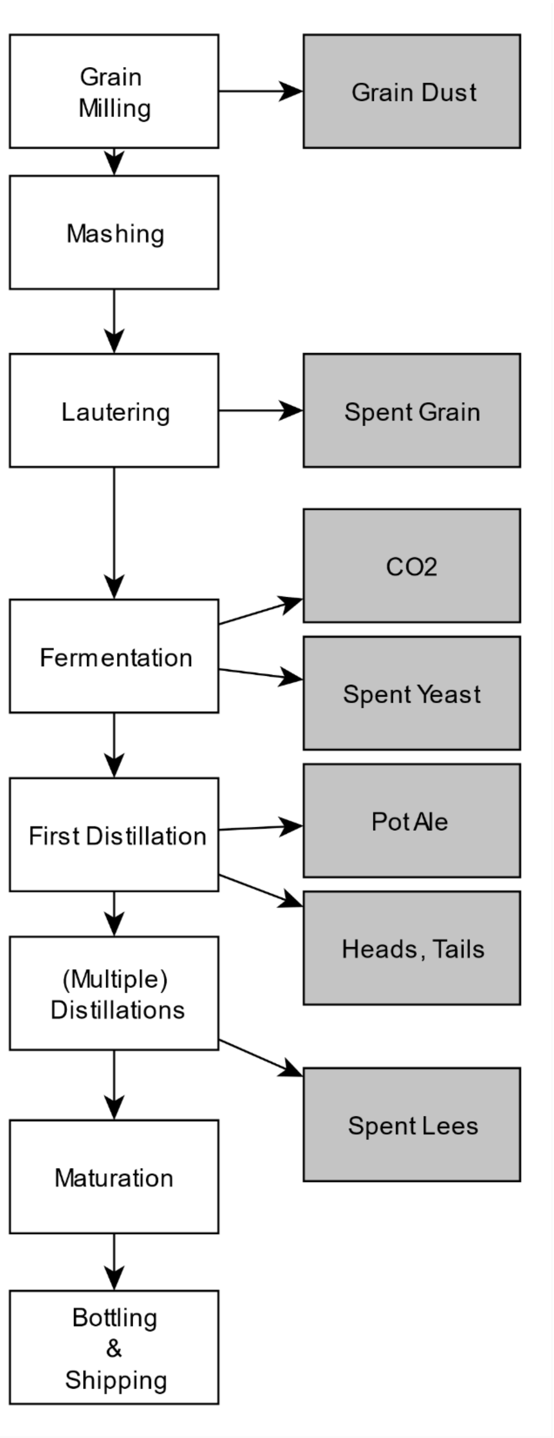

FIGURE 2: The whisky distillation process and the waste it generates

While it was not possible to find any information on the typical amounts of waste produced during the operation of a malt-based distillery, there was information available on the annual production of waste by Scottish malt and grain-based distilleries. However, this information was only presented as a percentage of utilization concerning the by-products made by the utilization of the corresponding waste streams. It was written that the annual production of all by-products in Scotland is around 500 kilotons of material (Bell, Farquhar & McDowell, 2019).

4 SPENT GRAIN

Spent grain is a co-product that remains both in brewing and distilling however, the exact volume is hard to determine and differs between the two processes. According to Anstruther et. al., 192 000 tons of dry matter were produced in 1999, and it follows due to the increase in breweries, distilleries and demand that this number has increased (Anstruther, Stewart, Russell, 2017, 252). It mainly consists of cellulose and residual moisture, but also contains starch residual sugars, proteins, lignin, and lipids.

TABLE 3: Chemical composition of BSG adapted from Lynch, 2016 (Lynch, 2016).

Component	**Kanauchi et al. 2001**	**Santos et al. 2003**	**Carvalheiro et al. 2004**	**Silva et al. 2004**
Hemicellulose (arabinoxylan)	21,8	n.d.	29.6	41.9
Cellulose	25,4	n.d.	21.9	25.3
Starch	n.d.	n.d.	n.d.	
Protein	24	31	24,6	n.d.
Lignin	11,9	16	21,7	16,9
Lipid	10,6	3,0-6,0	n.d.	
Ash	2,4	4,0	1,2	4,6
Phenolics	n.d.	1,7-2,0	n.d.	
	Mussatto and Roberto 2006	**Celus et al. 2006**	**Xiros et al. 2008**	**Jay et al. 2008**
Hemicellulose (arabinoxylan)	28,4	22,5	40	n.d.
Cellulose	16,8	0,3	12	31-33
Starch	n.d.	1	2,7	10-12
Protein	15,2	26,7	14,2	15-17
Lignin	27,8	n.d.	11,5	20-22
Lipid	n.d.	n.d.	13	6-8
Ash	4,6	3,3	3,3	n.d.
Phenolics	n.d.	2,0	2,0	1,0-1,5
	Robertson et al. 2010	**Waters et al. 2012**	**Meneses et al. 2013**	
Hemicellulose (arabinoxylan)	22-29	22,2	19,2	
Cellulose	n.d.	26,0	21,7	
Starch	2-8			
Protein	20-24	22,1	24,7	
Lignin	13-17	n.d.	19,4	
Lipid	n.d.			
Ash	n.d.	1,1	4,2	
Phenolics	0,7-0,9			

The composition of brewers spent grain has been a subject of research for quite some time, as can be seen by observing TABLE 3. Furthermore, the amino acid and mineral contents have also been analyzed and compared between spent grain, barely and barley malt. This is shown in TABLE 4 through 6. There are less essential amino acids than non-essential ones overall, and the amount of both kinds of amino acids increases during the brewing process.

TABLE 4: Non-essential amino acid contents of BSG, Malt and Barely compared. Adapted from Lynch, 2016.

Non-essential amino acids	**BSG**	**Malt**	**Barley**
Histidine	26,27	1,90	1,59
Glutamic acid	16,59	0,75	0,85
Aspartic acid	4,81	0,17	0,19
Valine	4,61	0,24	0,23
Arginine	4,51	0,23	0,21
Alanine	4,12	0,23	0,22
Serine	3,77	0,07	0,12
Tyrosine	2,57	0,14	0,14
Glycine	1,74	0,06	0,08
Asparagine	1,47	0,33	0,23
Glutamine	0,07	n.d.	n.d.

TABLE 5: Essential amino acid contents of BSG, Malt and Barely compared. Adapted from Lynch, 2016.

Essential amino acids	**BSG**	**Malt**	**Barley**
Lysine	14,31	3,69	2,52
Leucine	6,12	0,29	0,30
Phenylalanine	4,64	0,21	0,20
Isoleucine	3,31	0,17	0,17
Threonine	0,71	0,02	0,01
Tryptophan	0,14	n.d	0,01
Methionine	n.d.	n.d.	0,03

TABLE 6: Mineral content (% w/w) adapted from Lynch, 2016.

Mineral content	**BSG**	**MALT**	**BARLEY**
Phosphorus	0,46	0,27	0,24
Magnesium	0,24	0,09	0,08
Calcium	0,22	0,05	0,06
Silicon	0,14	0,06	0,05

The valorization of BSG requires treatment in almost all cases, as it is susceptible to microbial spoilage. An example of a pre-treatment plant has been described in literature, in which BSG is dried to reduce microbial growth and therefore, risk of spoilage (Cardona, Dávila, Rosenberg, 2016). To increase the efficiency of cellulose and xylose recovery, it is milled and subjected to acid hydrolysis in a sulfuric acid reactor. Finally, the sulfuric acid is neutralized by sodium hydroxate, resulting in sodium sulfate (Cardona, Dávila, Rosenberg, 2016, 55). Treatment methods for BSG can be separated between physical and biological methods. An example for such a physical method is the drying and pelletizing of BSG for heat generation purposes (Sperandio, Amoriello, Carbone, Fedrizzi, Monteleone, Tarangioli, Pagano, 2017). Biological methods for BSG valorization are numerous, have been described in literature and oftentimes require some sort of fermentation (Carradorini, George, Simet, Faour, 2017).

5 SPENT HOPS AND HOT TRUB

Hops are added to beer to impart bitterness and aroma. The addition of hops also aids the coagulation of proteins during brewing (Narziß & Gastl, 2017, 115). In most raw hop species, myrcene, α-humulene and caryophyllene occur in the highest concentrations (Nance, Setzer. 2011, 18-21). Spent hops are usually contained in the trub, a mixture of hops and debris which remains after the boiling process. There is significantly less hops consumed by the brewing process than grains, although the quantities are still notable, especially in bigger breweries or breweries that specialize in hop-heavy beers such as IPA’s.

A rather recent development was the use of pelletized hops instead of hop blossoms due to easier transport and storage. It must be noted that the antioxidant properties of pelletized hops are significantly different than their largely unprocessed counterparts. Warm storage of pelletized hops will substantially increase the antioxidant properties but will result in a loss of 50-60% α-acids while only 27-31% are lost when stored cold (Krofta, Mikyška, Hašková, 2008. 165). Since these acids are of great importance to the brewing process, hops are generally stored cold. Packing pelleted hops into multi-layer foils without air access will significantly reduce the effects of temperature on the antioxidant properties. The hop strains with the highest antioxidant properties so far have been found to be of the "Saaz" and "Spalter Select" varieties. (Krofta, Mikyška, Hašková, 2008). It must be considered that hop components undergo chemical changes after their utilization in breweries and may also do so when stored after extraction from the plant matter (Wilhelm, 2013). This could cause an increased difficulty when attempting to valorize used hops, as the chemical properties may have changed.

It is well established that hops can be used in medicinal ways, and some of the substances gained from hops have shown usage potential for medicinal applications. Estrogenic compounds derived from hops can be used in such a manner, while Xanthohumol can act as a novel anti-HIV-1 agent (Wang, Ding, Liu, Zheng, 2004; Chadwick, Nikolic, Burdette, Overk, Bolton, van Breemen, Richard, Fröhlich, Fong, Farnsworth, Pauli, 2004). Furthermore, the apoptosis-inducing agent desmethylxanthohumol can be synthesized from hop constituents (Diller, Herbert, Rose, Corazon, Henze, Prokop, 2005). Flavonoids acquired from hop cone preparations are useable against Staphylococcus aureus, even if the organism is resistant to methicillin (Bartmańska, Wałecka-Zacharska, Tronina, Popłoński, Sordon, Brzezowska, Bania, Huszcza, 2018). Other than the medicinal value of hops, it was found that some of their essential oils can be used to produce a biological pest repellant, some of these essential oils are listed in Table 8 (Bedini, Flamini, Girardi, Cosci, 2015). This last possibility can be used to benefit brewers, as they often have problems with pests such as mice in their grain storage, which can easily contaminate a complete batch of unused grains. Craft brewers oftentimes do not have the means or capital to build grain storage units that can withstand different types of pests. TABLE 7 shows the pest repellant that can be obtained from hops.

TABLE 7: Chemical Composition (%) of the spent hops essential oils that have been tested for pest repellent activity (Bedini, Flamini, Girardi, Cosci, 2015)

Constituents	**LRI**	**%**
Myrcene	993	24,2
α-Humulene	1,456	16,2
β-Caryophyllene	1,419	6,6
Linalool	1,101	1,9
Limonene	1,032	1,2

Myrcene has been researched as “Natural Base Chemical in Sustainable Chemistry”, as it was found that its unsaturated hydrocarbon skeleton can be treated with methods from petrochemistry (Behr & Johnen, 2009). While the essential oil fractions of hops are interesting, it is also beneficial to know their overall chemical composition, which is shown in TABLE 8. From this chemical composition, it becomes obvious that most of it is fiber and ash, while there is some phosphorous and ammonium nitrogen involved as well.

TABLE 8: Chemical composition of spent hops (adapted from Ziemiński, Romanowska & Kowalska , 2012)

Biomass component	**Unit**	**Spent Hops**
DM	[g/kg]	890,00
Ash	[g/kg]	102,35
COD	[g/kg]	115,72
Ammonium nitrogen	[g/kg]	23,47
Phosphorous	[g/kg]	5,51
Protein	[% DM]	20
Fiber, including:	[% DM]	57,7
Cellulose		19,6
Hemicellulose		12,5
Pectin		4,0
Lignin		21,0

There are several different methods for the extraction of hop compounds, such as the use of supercritical CO_2, which resulted in almost 99% degree of extraction for α-acids, 94,5% for β-acids and 96,5% for soft resins as well as fractions of other compounds (Hubert & Vitzhum, 1978). While the resulting extracts lack stability, CO_2 extraction is beneficial as no solvents which may remain in the extract are required (Hrnčič, Španinger, Košir, Knez, Bren, 2019).

6 SPENT HOP / SPENT TRUB EXTRACTION METHODS AND THEIR COMPARISON

Myrcene is one of the most abundant substances within the hops essential oil fraction. It is not exclusive to *Humulus Lupulus*, but can in fact be found in other plants as well, such as *Cannabis Stativa L*. Not only are these two plants biologically related, but they also share many essential oil components, therefore it may be suggested to compare corresponding research on extraction and valorization for both, as the methods might be similar. (Novak, 2001). While Myrcene has been used as a flavoring, such use is now discouraged in some countries due to the suspicions that it may be cancerogenic (Grosse, 2017). Instead, it may be used as an intermediary or starting material for the synthesis of different chemicals, such as epoxides, alcohols, esters, chlorides, acetates and more (Behr & Johnen, 2009). These applications make it a potential target for valorization, and the extraction of these compounds has been subject to research. One possibility is the use of steam distillation, and microwave assisted hydro distillation was shown to provide extract enriched with β-Myrcene (Sanz, Torres, Vilariño & Domínguez, 2019).

α-humulene is the second biggest fraction within the hop essential oils. It has anti-inflammatory properties, making it a candidate for pharmaceutical use (Karabín, Hudcová, Jelínek, Dostálek, 2016). It may be used in the prophylaxis or treatment of inflammatory pain or edema (Pianowski & Luiz, 2007). During the brewing process, compounds such as ferulic, gallic p-hydroxycoumaric, protocatechuic and caffeic acids, flavones and anthocyanidins are precipitated into the wort, along with a vast number of other substances (Moreira, Morais, Carvalho, Barros, Delerue-Matos, Guido, 2013). This explains why some of these substances are found in the hot trub as well. The chemical composition of hops has been researched for quite some time now, and new conclusions are drawn from recent research (Almaguer, Schönberger, Gastl, Ardent, Becker, 2014).

While the original intent of this work was to determine the different extraction methods used on spent hops, it was quickly found that the spent hops are withheld in the "hot trub". However, the focus will still lie on the spent hops and the possible compounds one could acquire from them, which have already been described previously. It was also found that extraction methods in the hemp industry are very similar since hemp and hops are related plants containing somewhat similar compounds.

Depending on the desired compounds, the extraction process must be designed accordingly. Extraction methods can differ in their degree of specificness, meaning that some may extract more compounds than are initially wanted. Supercritical CO2 extraction has extensively been researched for hops and hemp alike (Dzingelevičius, Maruška, Ragažinskienė, Obelevičius, 2011), (Porto, 2014).

Another method is the use of pressurized hot water extraction, which has been found to be more selective than subcritical ethanol or sequential extraction for the acquisition of Isoxanthohumol, which can further be refined into medicine (Gil-Ramírez, Mendiola, Arranz, Ruíz-Rodríguez, Reglero Ibáneñez, Marín, 2012). Solid-phase microextraction was done for raw volatiles to verify their variety. Liquified Dimethyl Ether has been found to be a green solvent for the extraction of Cannabis sativa seed oil (Subratti, Lalgee, Jalsa, 2019), and preliminary investigations in the laboratory have found that it may be a very promising solvent for spent hops as well, although more research is needed on this matter. Ultrasonic assisted microextraction has been used to acquire extract from hops, and it may be of use for spent hops as well.

In general, it may be said that the use of a solvent can be beneficial, but also problematic as the removal of said solvent must then be considered depending on how the extract is further utilized. Grilc & Dabrowski already described a system to extract bitter compounds from waste hop foliage in 1994, however this is a process that is tailored to raw hop foliage which has not undergone any treatment that one might subject the hop matter to in a brewery (Grilc, Dabrowski, 1994). The distillation process was already known long before such works (Hrnčič, 2019). Ethanol has been used as a solvent, but methanol, acetone, and ethyl acetate were also found effective in literature (Hrnčič, 2019).

Hop boiling times can differ significantly in breweries, therefor it would be interesting to experimentally determine the effect of boiling times on the hop constituents and the latter used extraction techniques. While it was desired to do so in laboratory scale, it was not advisable to continue these experimentations to any level of significant scientific proceeding due to the pandemic situation of early 2020. There has been work on the use of different solvents for the extraction of phenolic compounds from hops, which may be beneficial in future research (Mafakheri, Hamidoghli, 2019).

7 SPENT YEAST

Yeast is a eukaryotic unicellular micro fungus and a necessary ingredient to produce beer as well as whisky (Satyanaraya & Kunze, 2009). During the fermentation process, the yeast converts the sugars in the wort to alcohol. In general, distillers yeast requires a higher degree of attenuation than brewer's yeast, as the distiller wishes to convert as much sugar as possible (Stewart, Russell, Hill, 2013).

While the conversion of sugars it the most prominent function of yeast, it serves other purposes as well. For example, it imparts flavor by producing higher alcohols, esters, carbonyls, and sulfur compounds (Anstruther, Stewart, Russell, 2017). During the fermentation process the yeast multiplies and forms a significant amount of biomass. It is possible to reuse yeast up to six times; however, this practice carries the risk of spoilage due to contamination with foreign, unwanted organisms or due to natural mutations (LIFEYEAST, 2020). Research on the chemical composition of yeast extract has been conducted. TABLE 9 shows the amino acid content of such an extract (Podpora, Świderski, Sadowska, Rakowska, Wasiak-Zys, 2016).

TABLE 9: Amino acid content of two different extracts from brewers spent yeast (adapted from Podopra, 2016)

Amino Acid	**Total (g/100g)**		**Free amino acids (g/100g)**		**Free amino acids (%)**	
	A	**B**	**A**	**B**	**A**	**B**
Aspartic acid	6.18	5.79	2.23	2.36	36.08	40.76
Threonine	2.68	2.59	1.57	1.44	58.58	55.60
Serine	2.91	2.84	2.27	1.67	78.01	58.80
Glutamic acid	8.86	9.05	3.84	2.07	43.34	22.87
Glycine	2.91	2.94	1.53	1.01	52.58	34.35
Alanine	4.44	4.18	3.76	3.09	84.68	73.92
Cysteine	0.51	0.74	0.21	0.31	41.18	41.89
Valine	3.55	3.44	2.52	1.98	70.99	57.56
Methionine	0.90	0.90	0.74	0.62	82.22	68.89
Isoleucine	2.90	2.80	2.17	1.65	74.83	58.93
Leucine	4.10	4.09	3.52	2.98	85.85	72.86
Tyrosine	1.81	2.17	1.41	1.35	77.90	62.21
Phenylalanine	2.54	2.74	2.10	1.87	82.68	68.25
Histidine	1.34	1.36	1.00	0.64	75.63	47.06
Lysine	4.34	4.14		1.63		39.37
Arginine	3.02	2.73		1.43		52.38
Proline	2.81	3.79	1.78	2.00	63.35	52.77
Tryptophan	0.71	0.78	0.58	0.50	81.69	64.10
Total	56.51	57.07	35.28	28.60	62.43	50.11

Since the yeast cannot be reused indefinitely, it is considered spent yeast after having been sufficiently utilized. This co-product is rich in β-glucans, which can be utilized due to their rheological properties (Natakankitkul, 2016). When the cell wall of the yeast is disrupted, proteins, RNA, vitamins and minerals come available (Vieira, 2016). Furthermore, it can be utilized in the bioproduction of natural dyes (Rodrigues, 2019). Due to the high risk of spoilage, it is difficult to valorize yeast, albeit far from impossible. TABLE 10 shows different components of spent yeast and the possible valorization options thereof.

TABLE 10: Possible applications of brewers spent yeast (adapted from Lifeyeast, 2019)

Partially autolyzed yeast	**Yeast cell wall**	**Yeast Extract**	**Active peptides**
Fermentation activator (wine, beer)	Fermentation activator	Food/Beverage fortification (+lysine)	Food/Beverage supplementation, Antioxidant activity (CHP)
Fish nutrition	Feed: Immunomodulatory effect (β-glucan source)	Microbiological growth (Nitrogen source)	Hypoglucemic activity (active beverages)
	Feed: Pre-biotic effect	Brewers malt fortification	Hypotensive activity
		Flavor: Taste enhancing powder	

8 POT ALE

Pot ale is a caramelized organic turbid liquid residue with high COD and BOD, which is dark in color and contains solids (Walker, Graham, Peter, Wardlaw, Campbell, 2012). About 8 liters of pot ale are produced per liter of alcohol during the creation of whisky. Spent lees are like pot ale, but more dilute (Circular Economy, 2015). It contains water, yeast cells which are dead but intact, yeast cell residues such as cell wall material, polyphenols, phosphorus, sulfur, phytate and copper (Akunna, Walker, 2016). TABLE 11 shows the chemical composition of Pot Ale.

TABLE 11: Composition of pot ale and spent lees (Bennett, Walker, Murray, Akunna, Wardlaw, 2015)

Parameter	**Pot Ale**	**Spent Lees**
COD (mg/L)	50,000-75,000	1,500-4,00
BOD (mg/L)	25,000-35,000	500-2000
SO2-4	100-450	<40
PO3-4	150-600	<0.5
Cu (mg/L)	2-12	8-50
Cd (mg/L)	0-0.035	0
Al (mg/L)	0.03-0.150	0.01-0.08
Solid (%wt/wt)	4-7	0.02-0.175
Total Nitrogen (mg/L)	2,000-4,000	100-150

Another chemical analysis of pot ale is shown in TABLE 12. Here a significant difference in some of the measured parameters can be observed. This may be due to the raw materials having been grown and processed in alternative environments. The distillation process may also be cause for such significant difference, and other measurement techniques were used.

TABLE 12: Composition of pot ale after removal of initial solids (adapted from Uzukwu, Barraclough & Dionsi, 2017)

Parameter	**Unit**	**Value**
pH		3.7
COD	g/L	46
Ammonia	mgN/L	130
Phosphorous	mgP/L	647
Calcium	mg/L	87
Magnesium	mg/L	447
Copper	mg/L	0.25

Another interesting consideration when dealing which pot ale is the amount of acidic compounds it contains. It was found that fresh pot ale will contain approx. 100mg/L of acetic acid. However, with microbial activity it will turn sour, and this value can increase tenfold. While microbial spoilage is unwanted in most of the previously mentioned co-product streams, this increase in acetic acid may be useful if exploited. In Table 13, the acidic compounds of pot ale are displayed.

TABLE 13: Acids in pot ale (adapted from Walker et al, 2012)

Acid	**Approx. Amount in [mg/L]**
Acetic	1000
Lactic	120-150
Propanoic	100

The valorization of pot ale is being subject to research, which often includes some sort of anerobic digestion. Deproteinization by ion exchange chromatography is possible (Modinger, 2015), and the high amounts of nitrogen and phosphorus it contains can be recovered and used as fertilizer (Uzukwu et al, 2017).

9 VALORIZATION PATHWAYS

After having reviewed the brewing and distilling processes, the waste streams that emerge thereof and the compositions of the said streams, they can be categorized appropriately. This is shown in TABLE 14. Spent lees, being basically diluted pot ale, is omitted. To aid the interested reader in finding the mentioned applications within the source material, the author and year of publication is mentioned in the table, allowing to quickly compare it with the list of references. While the applications and their corresponding value are denoted as “Low” or “High”, it must be mentioned that this is a mere estimation based on the resulting product. Further research would be necessary to determine the applicability and economic suitability within the industry.

TABLE 14: The possible applications of the waste streams according to literature

Waste Stream	**Application**	**Value (Low / High)**	**Reference**
Spent Grain	Biofuel production	Low	Sperandio, G. Amoriello, T. Carbone, K. Fedrizzi, M. Monteleone, A. Tarangioli, S. Pagano, M. 2017.
	Animal feed	Low	Zebell, L. Deming, E. Heitland, J. 2016.
	Composting	Low	Zebell, L. Deming, E. Heitland, J. 2016.
	Sorption of heavy metals in wastewater treatment	Low	Low, K.S. Lee, C.K. Liew, S.C. 2000.
	Xylitol	High	Cardona, C. Dávila, J. Rosenberg, M. 2016. Carradorini, A. George, A. Simet, K. Faour, N. 2017.
	Lactic Acid	High	Carradorini, A. George, A. Simet, K. Faour, N. 2017. Radosavljević, M. Pejin, J. Kocić-Tanackov, S. Mladenović, D. Djukić-Vuković, A. Mojović, L. 2017.
	Fumaric Acid	High	Ibarruri, J. Cebrián, M. Hernández, I. 2019.
	Lipids	High	Patel, A. Mikes, F. Bühler, S. Matsakas, L. 2018.
	Polyhydroxybutyrate	High	Cardona, C.A. Dávila, J.A. Rosenberg, M. 2016.
	Polylactic acid	High	Carradorini, A. George, A. Simet, K. Faour, N. 2017.
Spent Hops / Trub	Addition to animal feed after debittering	Low	Vandamme, E.J. Nigam, P.S. Pandey, A. 2009.
	Polyphenols	High	Tišma, M. Šalić, A. Planinić, M. Zelić, B. Potočnik, M. Šelo, G. Bucić-Kojić, A. 2020
	Pest Repellant	High	Bedini, S. Flamini, G. Girardi, J. Cosci, F. Barbara, C. 2015.
	Pharmaceutical Agents	High	Wang, Q. Ding, Z.H., Liu, J.K. Zheng, Y.T. 2004.
	Antioxidant	High	Mafakheri, M. Hamidoghli, Y. 2019.

(continues)

TABLE 14. (continues).

Spent Yeast	Nutraceuticals	Low	Sadowska, A. Podpora, B. Swiderski, F. Rakowska, R and Wasiak-Zys, G. 2016. LIFEYEAST. 2019.
	Fermentation Activation	Low	LIFEYEAST, 2019.
	β-glucan source for animal feed	Low	LIFEYEAST, 2019.
	Pre-biotic for animal feed	Low	LIFEYEAST, 2019.
	Biosorption of copper and lead	Low	Han, R. Li, H. Li, Y. Zhang, J. Xiao, H. Shi, J. 2006.
	Protein source	High	Sadowska, A. Podpora, B. Swiderski, F. Rakowska, R and Wasiak-Zys, G. 2016.
	Microelement source	High	Vieira, E. Carvalho, J. Pinto, E. Cunha, S. Almeida, A. Ferreira, I. 2016.
	Taste enhancement	High	LIFEYEAST, 2019.
	Medicinal and Nutritional applications	High	LIFEYEAST, 2019.
	Cosmetics	High	Natakankitkul, S. Homdok, P. Wadee, P. Krisdaphong, T. Toida, T. 2016
	Natural Dyes	High	Rodrigues, T. Schueler, T. da Silva, A. Sérvulo, E. Oliveira, F. 2019.
Pot Ale	Animal feed addition as pot ale syrup	Low	Uzukwu, C. Barraclough, J. Dionisi, D. 2017.
	Protein source	High	Modinger, J. 2015.
	Possible medium for bio-reactors in pigment production	High	McNerney, C. 2019.

To further the understanding of the valorization options of the shown waste streams, a review of the technological processes related is in order. These differ depending on the raw material used, the preparation thereof for further processing and finally the resulting products. It should be noted that these technological showcases are only a few of the myriad of available options, as the underlying research is ongoing in all these fields. At the time of writing this work, new possibilities are already being studied by the international academic community.

9.1 Case Study: Production of laccase and polyphenols from brewers spent grain

In the works of Tišma et al (Tišma, Šalić, Planinić, Zelić, Potočnik, Šelo, Bucić-Kojić, 2020), Brewers Spent Grain is used to produce valuable compounds such as laccase and polyphenols. This is interesting as it results in two valuable products, showing that the valorization process is not limited to only one product. In order to do so, fermentation with *Trametes Versicolor* was utilized. Since other microbial or fungal growth was unwanted, the sample of BSG was autoclaved until sterile, dried, milled and finally innoculated with the fungus. For 14 days, aseptic samples were taken and analyzed for their laccase content. After 14 days, 35% of the BSG biomass was degraded while laccase activity increased significantly.

The total polyphenol concentration also increased. The corresponding researchers note that the difference in BSG parameters could alter the results of their experiments and suggest redoing the experiment with samples of raw material from different breweries. While the sucrose and glucose content of the BSG degraded over time, the fructose content increased ca. 30-fold. This can be considered a success in valorization.

9.2 Pharmaceutical Agents from Spent Hops

Bartmańska et al (2018) have researched the antimicrobial properties of spent hop extracts. In order to do so, they used four different organic solvents to acquire extracts from spent hop material. The said spent hop material was not used in the brewing itself. Instead, the brewers opted to use supercritical CO_2-Extraction of the hop cones. This is done mostly by bigger breweries, but recently home and small breweries have started to use hop extracts as well. Machines for hop extraction in the brewery exist (Baskette, R), although most breweries buy readymade hop extract from specialized manufacturers. Either way, spent hops are produced and the CO_2 extraction process does not remove all of the hop compounds.

Therefore, Bartmańska et al (2018) opted to use spent hops that have already undergone supercritical extraction. Using four solvents with different polarities, 250g of the hop material was used with 1 liter of solvent for each sample. After removal of the solid phase by use of a paper filter, the remaining material was again washed with 400ml of solvent. The solvent was then evaporated in vacuum with a temperature remaining under 50°C. Afterwards the samples were stored at -20°C and subsequentially analyzed for their Xanthohumol content. The extracts were then further purified using HPLC.

Using different types of pathogens, such as Staphylococcus aureus, Salmonella typhimurium and Listeria monocytogenes in agar diffusions, Bartmańska et al (2018) were able to illuminate the antimicrobial effects of the hop extracts. It was found that methanol is the solvent of choice for crude extract of spent hops, as increased polarity of the solvent increases yield. However, this seems to be due to the supercritical processing the hops were subjected previous to the experiment.

While none of the extracted compounds inhibited Listeria monocytogenes, only aurone was effective against Salmonella Typhimurium, but all of them were shown to be active against Staphylococcus. Antifungal activity was displayed against Fusarium and Aspergillus species. However, the effectiveness of the crude extract against the fungus was highly dependent on the solvent used for preparation of the crude extract. In conclusion, spent hop extracts may be useful as food preservatives and as a form of green pesticide against plant pathogens.

9.3 Case Study: Flame retardent from spent yeast

While spent yeast is mostly popular due to its high content of beta glucans, other constituents can be valorized as well. One such example is the extraction of nucleic acids which are then further refined into flame retardents (Bosco, F. Casale, A. Gribaudo, G. Mollea, C. Malucelli, G. 2017). In this case the yeast cells are centrifuged after being stored at 4°C for more than six months. Afterwards, they are washed with bi-distilled water. Another sample was immediately centrifuged and washed with bi-distilled water. 1mL of buffer was added to 8 mg of yeast pellet weight and frozen at -20°C for 24 hours. Thawing occurred three times at 65°C for 15 minutes. Using 1- and 2-mm glass beads, the cells were mechanically disrupted, using 5g of glass beads for each 1 mg of yeast. Ball milling was performed for 10 minutes and the cell debris removed by centrifugation. Micro- and ultrafiltration were carried out although additional chemical purification was avoided. Bosco et al varied the parameters for their extraction by using different types of buffers and milling times. Autolyzed and fresh yeast were subjected to flammability tests. Nucleic Acids that were applied to cotton fabric have shown to be self-extinguishing. This makes nucleic acids from spent yeast a prime candidate for flame retardant products, as the material is biological and environmentally friendlier than its synthetic counterparts.

9.4 Using microalgae and LED phobioreactors in conjection with pot ale

McNerney has researched the applications of pot ale quite extensively. One of the experiments described uses pot ale in an LED-powered bioreactor to produce carotenoid pigments. Since the pot ale did not have the required pH value for microorganism growth, it was mixed with naturally occurring sea water. Using the algae Synechocytias sp, further investigation revealed that 50% diluted pot ale does not hinder light absorbance. Any less diluted pot ale showed signs of decreased light absorbance. It was found that the pH of pot ale is a major limiting factor concerning cyanobacterial growth.

This means that pot ale must be diluted to be used as a nutrient source for microalgae, although dilution naturally means that less nutrients will be available. While it is possible to grow microalgae in pot ale, it is difficult and not economic with current technology. The main challenges in this case seem to be the turbidity of the pot ale limiting light absorption and the considerable amount of copper dissolved in pot ale, which is toxic to cyanobacteria. If these challenges can be overcome, either by preparing the pot ale to remain clear without removing the nutrients, or by engineering a cyanobacterium that thrives in such conditions, pot ale may be a suitable growth media in the bioproduction of carotenoid pigments. (McNerny, 2019).

9 CONCLUSION

Significant amounts of solid and liquid wastes are disposed in the alcoholic beverage industry. These include plant material such as spent grains or hot trub, but also fungal material in the form of spent yeast. Liquids such as pot ale also must be considered, although the process of valorizing these is different and seems difficult. The production processes are indeed similar for beer and whisky, and valorization especially in the field of spent grain can be done with comparable technologies. The biochemical makeup of these waste streams contains a great number of valuable compounds that simply must be extracted and purified in order to gain value. Especially supercritical extraction processes have received much attention in the academic world, but solvent based extraction techniques, especially with the use of green solvents, is on the rise. On the topic of spent hops there has not been as much research, but the limited available results show great promise regarding valorization. With some countries applying new legislations, new ingredients are added to beer production which will in turn affect the co-product streams in the brewing industry.

One such ingredient is Cannabis, which exhibits surprising similarity to hops, albeit still being different. It is important to observe this use of new ingredients closely when considering valorization, as they may require alterations to the valorization processes. However, it may also be possible to combine valorization processes to deal with brewing waste as well as waste from the hemp industries. While traditionally most of the waste from breweries and distilleries was used as animal feed or fertilizer, the industry has outgrown these possibilities and new valorization pathways must be developed. While much focus is laid on the raw ingredients of the products and their subsequent valorization, one should also consider that distilleries and breweries alike use a myriad of cleaning chemicals in order to keep their facilities sterile. These chemicals are also a danger to the environment but revalorizing them would be difficult. Finally, carbon dioxide is produced in large quantities due to fermentation. The ability to capture, purify and re-use carbon dioxide from this process would greatly reduce costs for breweries.

On the topic of valorization technology, almost all the presented methods are biochemical in nature and require fermentation with different microorganisms. In most cases, some sort of fungi is used to acquire valuable compounds. However, pre-treatment of the raw waste streams is also still required. Once the waste stream has been treated and turned into a base compound for other material, the applications can range from simple energy purposes to more complex utilizations, such as environmentally friendly flame retardant in the textile industry. This strong focus on environmentally friendly solutions to known problems is a benefit towards the valorization of biological waste streams.

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