fermentation

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Review

A Review on the Source of Lipids and Their Interactions during Beer Fermentation that Affect Beer Quality

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Abstract: The presence of lipids in wort and beer are important due to their influence on yeast metabolism and beer quality. Barley lipids have long been considered to have adverse effects on beer quality where some long-chain fatty acids are associated with high flavour potential. In addition, beer foam stability can be influenced by the concentration of lipids as well as other factors such as hop acids (e.g., iso-α-acids), proteins, polysaccharides and the presence of metal ions (e.g., nickel). Lipids can also influence yeast protease activity as well as the production of ethanol. This review provides an overview of the effect of climate change on the chemical composition of barley in relation to lipids and the influence of lipids in the process of this raw material in order to produce beer.

Keywords: beer; fermentation; lipids; fatty acids; foam; quality

1. Introduction

Fermentation is a remarkably complex system and there has been considerable research focused on the effects of different levels of interactions among yeast and chemical compounds during the fermentation process of beer and other beverages [1].

The Food and Agriculture Organization (FAO) report the annual global cereal production is approximately 2.5 billion tonnes, which indicates the importance of cereal grains to society [2]. Cereal grains are used for food production such as bread making and brewing. They are also used as raw material by the biofuel industry and several other industries such as polymer productions [3]. In beer brewing much attention is given to the malt extract (e.g., quality and composition) because it determines the amount of beer that can be produced [1,4]. In particular, fermentability and malting properties are characteristics of the malting grain and they are key features used by the brewing industry as well as in breeding programs to develop improved malting crop varieties [1,2,5].

Global demand for cereal grains is anticipated to double by 2050 [3,6,7]. The effects of climate change are a challenge for crop production systems and it has been recognised that climate change might induce a temperature increase up to 2 °C or more in cereal growing regions, which will negatively impact crop production as well as the chemical composition of the grain [6,7]. Climate stresses affect plant metabolism, cellular homeostasis, growth development and the cause the uncoupling of physiological

and biochemical processes [8–12]. For example, high temperatures disrupt photosynthesis and increase photorespiration thereby altering the normal homeostasis of plant cells [8–12]. Therefore, climate change may contribute to losses in crop yield and potentially change the chemical composition and technological properties of the grain and concomitantly the nutritional value, flavour and aroma of the end product [8,9]. In addition, the potential for undesirable contaminants such as acrylamide, furan and trans fatty acids being formed during industrial processing is determined by the composition of the starting grain [8,9]. It has become clear that understanding the chemical composition of the raw material (barley) and the optimal growing environment required to produce ideal grain for industrial use, is essential to determine changes in the properties of the raw materials used to produce beer. Which will then dictate what technological adjustments that may be necessary to the brewing process to ensure the quality of the final product.

This review provides an overview of the effect of climate on the chemical composition of barley in relation to lipids and the influence of lipids in the process of this malting material in the production of beer.

2. Barley Grain

Barley is the main grain used in the production of beer. Barley, *Hordeum vulgare* is an annual monocotyledonous herb belonging to the Poaceae family [1]. Barley cultivars have been selected to express desirable features that fulfil the specifications for beer production (e.g., protein, amylose, amylopectin) [1,4]. Barley grown for the malt industry is limited to cultivars of high starch content, low moisture, adequate protein levels and high rate of germination [1,4].

The main components of barley grain are starch (62.7%), proteins (11.1%), lipids (2.1%), fibre components (9.7%), moisture (12.1%) and minerals (2.3%) [1,4]. The husk completely encases the grain separating living tissue from the outside environment. It protects the grain from physical damage and acts as a barrier to the diffusion of gases, water and solutes. The endosperm is the main tissue of the grain and is composed mostly of starch granules (1.7–2.5 μm to larger 22.5–47.5 μm granules) that are embedded in a protein matrix [1,4].

In addition to proteins and starch, barley contains polar lipids and fatty acids which are closely associated with polysaccharides, creating amylose-lipid complexes. In particular, amylose is a linear molecule formed by D-glucose residues linked by α(1–4) bonds in a helicoidal structure [13–15]. The molecule has hydrophobic characteristics which are important in the biological medium because it allows complexation with free fatty acids, alcohols and iodine. Amylopectin is another important molecule in the starch matrix. It is a highly branched polymer molecule formed by D-glucose units linked in an α(1–4) fashion permitting the branched like structure [13–15].

3. Effect of Climate on Grain Composition and Lipids

Olesen and collaborators [12] reported how climate affects cereal crop production. The authors highlighted a number of climate effects directly and indirectly including how an elevated CO_2 concentration in the atmosphere impacts directly on crop productivity and resource use efficiencies, affects crop development and growth, climate and how climate change can impact directly, damaging plants through extreme events such as extreme heat waves, drought, frost, hail and flooding [12]. On the other hand, indirect effects include shifts in the suitability of different cultivars for a particular growing climate, observed changes in crop nutrition requirements and the occurrence of weeds, pests and diseases and degradation of the environment such as soil erosion and pollution including; nitrate leaching [12].

The effects of elevated carbon dioxide and heatwaves during grain filling have been also reported [11]. Researchers identified that wheat cultivars had sustained damage during the grain filling period but mixed responses were observed for different cultivars, some showing increased, maintained and decreased protein concentrations [11]. Research has reported that the synthesis of β-glucans, the main structural component of endosperm cell walls, is reduced by water stress episodes

during the grain filling period [11]. A recent study showed that besides genetic differences between barley strains, the growing climate conditions can alter beer sensory descriptors, which the researchers concluded was most likely related to the malting performance of the barley [16]. Another study showed that higher temperatures and rainfall (relative to other areas in Tibet) during growth and development, can result in the annealing of starches and enhanced amylose–lipid complex formation, which has implications for pasting properties of the grain [17].

Bravi and associates investigated the lipid and fatty acid profile of genetically different barley varieties as well as the relationship between barley quality and malt [18]. The researchers reported a negative correlation between total lipid content and quality of the malt. The study involved producing malt from spring and winter varieties in the laboratory, when these malts were tested a decrease in oleic content and an increase in linoleic acid was observed in the spring varieties [18]. However, the winter variety had palmitic and linoleic acids that did not vary during the malting process. The researchers applied principal component analysis (PCA) to differentiate between the samples and concluded that the winter variety sample had lower polyunsaturated fatty acids [18]. They concluded that this variety could be used to improve the shelf life and beer quality [18]. The sample size for this experiment was small (1 winter and 4 spring varieties) but showed important differences between winter and spring varieties [18].

Evans and colleagues (2012) observed that the malt from different barley varieties could result in a range of different fatty acids in the wort [19]. The dominant fatty acids in barley grain, wort and brewed beer are palmitic acid (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) [19]. Mash temperature above 65 °C substantially increased the level of total fatty acids extracted with notable increases in linoleic and linolenic and decreases in palmitic acid [19]. The researchers suggested the extra lipids were extracted from the embryo or the aleurone layer of the grain and did not originate from internal starch lipids [19]. A recent study showed that both genetic differences between barley and the growing climate conditions can alter beer sensory descriptors, which the researchers concluded was most likely related to the malting performance of the barley [20].

Brennan and associates (1995) used scanning electron microscopy to identify structural differences between good and poor malting grains in winter and spring cultivars [21]. The researchers concluded that good malting varieties have weak associations between starch granules and the protein matrix [21]. The authors theorised that, for the poor malting lines, hydrolytic enzymes had restricted access to starch granules within the grain due to higher protein associations blocking the substrate [21]. The presence of lipids and fatty acids may also interfere with malting properties. For example, phospholipids raise the gelatinization temperature of starch granules which interferes with the malt extraction process [22–24]. Therefore, it is plausible to assume brewers may use higher temperatures or increase the heating time to increase the starch extraction process, which also increases the amount of lipids extracted [22–24].

In a recent study, Cozzolino and associates (2014) assessed the link between water uptake ability and the lipid profile of barley grain from Australia [25]. The researchers reported that oleic (18:1-n9) acid had a positive effect whereas long-chain fatty acids, arachidic (20:0) and lignoceric (24:0) acids had a negative effect on the water uptake of the grain, a first step of the malting process [25]. In a separate study, the same authors (2015) investigated the relationship between industry standard malt quality measurements (e.g., hot water extraction and apparent attenuation limit) and the fatty acid content of barley grain, malt and wort [26]. Results from this study alluded to lipids and fatty acids interfering with industry standard measurements, which only measure starch using density or sugar content measured as % Brix [26]. Industries relying on these measurements may be misled by a favourable reading because a high lipid content is interfering with the reading [25,26].

The relationship between starch structural features (e.g., protein matrix and amylose-lipid complexes) and other grain parameters (e.g., grain size, yield, etc.), are essential in determining the processing and manufacture of barley derived food and beverage products [27–31]. Yet, investigations on the molecular structure of barley starches and its relationships to proteins or more specifically, lipids are uncommon in the literature. The importance of lipids is likely to be overlooked in beer

brewing, because malt quality barley is often selected based on qualities such as protein content or grain size which are not related to lipids and their effects during fermentation and therefore their contribution ignored when assigning fault if the beer's quality is substandard [27–31]. A recent study investigated the features of Australian grown barley and identified that the protein content in malt quality barley is negatively correlated with grain size and the starch molecular structures, defined as the ratio between amylose and amylopectin [27–31]. Although protein and total starch are routinely measured for malt grade barley, limited analysis is performed to determine the content and composition of the starch structure or lipid profile of the grain which, as discussed previously, contribute to food and beverage processing [27–31].

Through a series of studies Yang and associates used modern breeding techniques to alter several carbohydrate traits in cool-climate hull-less barley grain varieties [32–34]. They found that by altering carbohydrate traits, the protein supply from barley cultivars were also significant changed [32–34]. These authors further studied the casual relationship and characterised the molecular structural features of a newly developed hull less barley cultivar in relation to nutrient utilization and availability [32–34].

4. Lipids during Fermentation Affecting the Beer Flavour Profile and Quality

Beer quality and yeast metabolism during fermentation of wort can be affected and modulated by the concentration of lipids present in the grain and wort [1,4]. While a number of long-chain fatty acids have been reported as precursors for characteristic flavours there is evidence that certain lipids may adversely affect beer quality [1,4,18,35]. For instance, flavours associated with beer aging have been attributed to the oxidative degradation of linoleic and linolenic acids during the fermentation process. One example of this is the presence of trans-2-nonenal, one of the more commonly recognized flavour compounds derived from lipid oxidation possessing a flavour threshold of 0.1 µg/L [18,22,28,36]. Temperature, sunlight exposure and storage time are the prime factors in the production of stale-flavoured breakdown products [18,24,28]. Moreover, some authors report the contribution of non-oxidised lipids, lipid derived free radicals and lipid oxidation products on the formation of Strecker aldehydes such as benzaldehyde, 2-methylpropanal and methional [18,23,24,28]. The ratio between unsaturated and saturated fatty acids is associated with the gushing phenomenon, with unsaturated fatty acids acting as gushing-suppressors and saturated fatty acids acting as gushing-promoters [18,23,24,28,37,38]. The activation of yeast cell growth under the anaerobic conditions of the wort fermentation process require long-chain unsaturated fatty acids. Consequently, lipids significantly influence the extent and speed of fermentation, concurrently increasing organic acid levels within the product contributing to sensory and flavour effects [18,28,37,38].

The composition of the malt and the lauter turbidity can result in the formation of multiple forms of lipids in the brewing process, such as simple lipids (e.g., triacylglycerols, diacylglycerols, monoacylglycerols and free fatty acids), complex lipids (e.g., phospholipids and sphingolipids) and covalently bound lipids [18,28,37,38]. The presence of these lipids during the brewing process has both positive and negative influences on the fermentation. For instance, some authors have reported improvements to fermentation performance without impacting the final product quality using worts with higher linoleic acid content [18,28,37,38]. Bravi and collaborators reported that the most representative fatty acids during the fermentation of the wort were palmitic and stearic acid [18]. These authors did observe that unsaturated fatty acids, oleic and linoleic acids were also present, with linolenic acid appearing in the 9° Plato wort, which is produced by mixing several malts including Pilsner, Special B, Chocolate malt, Carapils and Carahell [18,28]. A positive correlation between the specific density of beer wort samples and their fatty acid content was reported [18,28]. Moreover, the composition of fatty acids in the wort was explained by considering the variety of malt used and the potential of lipolytic activity, which involves the action of lipase, lipoxygenase and hydroperoxy fatty acid catalysing enzymes, during wort production [18,28,39–41].

Several beer quality parameters, such as aroma and taste descriptors, flavours and yeast metabolism are influenced by the wort and fatty acids within the wort. Long-chain unsaturated fatty acids have been demonstrated to significantly influence yeast starter cultures, both in terms of their growth and metabolism, however the sugar and protein content of the wort will also impact the fermentation performance [38,42]. The vastly different compositions in free fatty acids of beer worts, some of which have higher content of unsaturated fatty acids than others, may cause major oxidative damage, form undesirable flavours and have a damaging effect on foam stability of the final product [18,28,42].

5. Lipids and Their Role in Beer Foam

Beer foam is one of the most important factors in terms of beer quality [38]. The stability of beer foam is mostly due to hydrophobic interactions between a variety of species including polypeptides, polysaccharides, iso-α-acids and lipids, whereas competing surfactants and chaotropic agents (e.g., ethanol, magnesium chloride) disrupt this stability [38,43]. Notably, the dynamics of the adsorption of amphipathic species and foam active compounds into foam lamellae, are not fully realised due to the difficulties in studying a heterogeneous, multi-phase and transient mixture.

It is well established that lipid binding proteins, such as protein Z (serpins), lipid transfer protein 1 (LTP1) and puroindoline/hordoindoline proteins of wheat and barley malts contributed significantly to the formation and stability of beer foam [38]. Unlike puroindoline and hordoindoline, Protein Z and LTP1 proteins are capable of tolerating the brewing processes high temperatures maintaining some of their binding activity. The LTP1 proteins, such as glycosylation, often experience alteration during the brewing process as a consequence of their exposure to the resultant elevated temperatures [44–60]. Post-translational modification of LTP1 proteins, occur due to the action of a lipid-like adduct covalently bound to it, leads to the formation of LTP1b proteins [46–60]. For example, the production of a hydroperoxide, by the oxygenation of linoleic acid resulted in the subsequent dehydration of this molecule to form α-ketol adduct (9-hydroxy-10-oxo-12(Z)-octadecenoic acid) [46–60].

LTP1 proteins are also associated with multiple biological functions such as lipid transfer between lipid bilayers, seed development and pathogen control [46–60]. Multiple authors speculate that the unique lipid binding affinity of LTP1 proteins, shields the extracellular membrane of grains repelling damaging microbes [46–60]. This highlights further the importance of LTP1 proteins to the brewing industry as the synergistic interactions of such proteins and lipids significantly alter the food and beverage production processes, because of alterations in grain composition, brought about through environmental and or genetic factors. For example, LTP1 has been shown to have inhibitory effects on the common lager brewing yeast, *Saccharomyces pastorianus* and the ale brewing yeast, *S. cerevisiae*, as well as some fungal pathogens occurring in the field, which can be detrimental to the malting and fermentation stages of beer brewing [46–61].

Due to LTP1s resistance to high temperatures, its binding activity remains high following the mashing process and thus is still viable during fermentation [46–60]. The peptides are thus not only important to brewers as a major contributor towards foam formation and stability but because they can also influence fermentation [46–60]. It has been reported that neither LTP1 nor LTP1b denature completely at elevated temperatures (up to 100 °C), greater than normal mashing temperatures consequently it is imperative that the proteins bind activity throughout the brewing process should be considered [46–60]. LTP1 proteins account for 5–10% of all soluble proteins in grains and are found in wheat and barley grains used for malting. Consequently, highlighting the necessity to optimise traditional beer purification methods for barley LTP1 and LTP1b proteins [46–61].

6. Concluding Remarks

The presence of lipids in wort and beer are important due to their influence on yeast growth and metabolism as well as in different aspects of beer quality (e.g., foam stability). Barley lipids have long been considered to have adverse effects on beer quality, despite long- chain fatty acids possessing

high flavour potential. In addition, beer foam stability can be influenced by the presence and profile of fatty acids as well as other factors such as hop acids, proteins, polysaccharides and metal ions. Overall, lipids originating from the raw materials used to produce beer are very important, yet their full role in beer production and fermentation processes has not been fully elucidated. Understanding the nuances related to the origin of barley grains and what specific influences they have on the beer brewing process may provide new information for improving and controlling sensory factors, flavours and blending regimes to optimize quality.

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