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Review

The production of gluten-free beer: Degradation of hordeins during malting and brewing and the application of modern process technology focusing on endogenous malt peptidases

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ABSTRACT

Background: Celiac disease (CD) is a potentially life-threatening condition affecting 1% of the Western Europe population. It is caused by a T-cell-mediated inflammatory autoimmune response of the small intestine. Patients suffering from CD have to adopt a lifelong gluten-free (GF) diet. To increase their living standards, several techniques to produce GF beer have been established. More recently, endogenous malt peptidases have come into focus.

Scope and approach: This review critically discusses current methods to produce GF beer from GF cereals and pseudocereals, traditional and molecular breeding, and exogenous enzymes. Focus is also placed on the modification of immunogenic gluten proteins during malting and brewing and the application of gluten-specific malt peptidases such as endoprotease B2 for detoxification of hordein, including proteomic characterization and limitations of quantitative analysis by ELISA.

Key findings and conclusions: B-, C-, D-, and γ-hordeins undergo partial solubilization, proteolysis, physical retention, gradual unfolding, and denaturation during malting and brewing. Endoprotease B2 has been shown to degrade these fractions. Although it has not been used for food detoxification, gluten-specific peptidases can degrade gluten in beer below the postulated 20 mg/kg. Alternative methods have various disadvantages, such as process modification and deviating product quality, as well as discordance regarding national legislations and consumer acceptance. In terms of breeding, secondary mutations can occur and the procedure is time-consuming. Given that gluten-specific peptidases occur naturally in the grain itself, are simple to extract, the technology of malting is well established, and no genetic engineering is necessary, they are a promising alternative to current process technologies.

1. Introduction

Celiac disease (CD) is a chronic inflammatory reaction in the small intestine triggered by the ingestion of immunogenic prolamin and glutelin peptides derived from barley, wheat, and rye. This autoimmune disease leads to a reduction of the intestinal villi, ultimately leading to total atrophy. Celiacs show symptoms ranging from diarrhea, fatigue, and vomiting to dermatitis and suffer reduced uptake of vitamins and minerals. Additionally, an increased risk of diabetes, osteoporosis and life-threatening small bowel cancer was detected (Rostom, Murray, & Kagnoff, 2006). The only treatment for CD is lifelong abstinence from gluten-containing products that are made from barley, wheat, or rye and contain more than 20 mg of gluten per kilogram. Gluten is a collective term for prolamins and glutelins in wheat named gliadin and glutenin, rye termed secalin, and barley called hordein (Belitz, Grosch, & Schieberle, 2007), which can be further subdivided according to molecular weight, number of amino acids (AA), and repetitive AA residues. It is still controversial whether the prolamin and glutelin fractions of oat (avenin) are suitable for celiacs, with only a few clinical studies having been published and the results, also discussed in different reviews, being contradictory (Fric, Gabrovska, &

Abbreviations: AA, amino acid; ACEi, angiotensin-converting enzyme inhibitory peptides; AN-PEP, prolyl endopeptidase from *Aspergillus niger*; CD, celiac disease; CQP, *Chenopodium quinoa* polysaccharide; EPA, endoprotease A; EPB, endoprotease B2; FAN, free amino nitrogen; GF, gluten-free; HMW, high-molecular-weight; iTRAQ, isobaric tags for relative and absolute quantitation; LMW, low-molecular-weight; mg/kg, mg of gluten per kilogram; MMW, medium-molecular-weight; mTG, microbial transglutaminase; PTC-digest, peptic-tryptic-chymotryptic digest; RNAi, RNA interference; TBI, thiobarbituric acid index; TG, transglutaminase.

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Nevoral, 2011; Haboubi, Taylor, & Jones, 2006). The main risk with oats appears to be the high risk of contamination with gluten-containing materials. The prevalence of CD in Western Europe has recently been determined to be 1:100 (Lebwohl, Ludvigsson, & Green, 2015), but the validity of this value is unclear because of silent forms and low clinical rates of detection. Beer labeled *gluten-free* contains less than or equal to 20 mg/kg according to Codex Stan. 118–1979 and most national legislations such as in Europe, where regulation EU 1169/2011 is valid based on Codex Stan. Regulations that deviate from this include the Federal Alcohol Administration Act (Alcohol and Tobacco Tax and Trade Bureau, 2014), valid for the US market, where beer made of barley, rye, wheat, or their derivatives is not allowed to be labeled GF in general (United States Food and Drug Administration, 2013); another of these is the Food Standards Code of Australia and New Zealand, where food must not contain any detectable traces of gluten (Australia New Zealand Food Standards Code, 2017). According to Codex Stan. 118–1979, the method for gluten determination is the enzyme-linked immunoassay (ELISA) R5 Mendez method. Here, for analysis of hydrolyzed hordeins as they occur in processed food, for example, beer, competitive ELISA is recommended as it needs only one epitope for antibody-antigen reaction, in contrast to sandwich ELISA, which needs two epitopes. The gluten content measured in cereal-derived, fermented beverages such as beer does strongly vary depending on the type, variety, and crop year of the cereal as well as the malting and brewing procedure. To manufacture GF beer in a reproducible way, which has $\leq$20 mg/kg gluten and conforms to the above-mentioned legislation, a wide range of techniques has been established to produce GF beer, primarily focusing on either the use of GF grains or the degradation of gluten-containing grains during the process. A new technique, the use of endogenous malt enzymes to produce gluten-free beer, has emerged recently and is a promising alternative. This review provides an overview of the modification of hordein during malting and brewing, as barley is the most widely used cereal for brewing, including its analysis. Focus is also placed on the application of enzymes for the detoxification of hordeins in beer, focusing on cereal-derived enzymes, especially endoprotease B2 (EPB). This paper also includes a critical discussion of recently established techniques to produce GF beer from GF cereals and pseudocereals, traditional and molecular breeding, the use of exogenous enzymes, and the limitations of gluten analysis using ELISA.

2. Techniques for the production of GF beer from GF raw materials

A wide range of methods to produce GF beers have been established. These can mainly be distinguished by the category of raw materials applied, using naturally GF cereals, pseudocereals, or adjuncts, as well as classical-, and molecular breeding technologies. Alternatively, process engineering applied has to be modified to degrade gluten when using traditional, non-GF raw materials. Here, both the malting and brewing procedures have to be investigated on their gluten degrading potential or endogenous, or exogenous enzymes have to be applied. Fig. 1 gives an overview of the available options, which will be critically discussed and explained in detail hereinafter.

2.1. Alternative raw materials – GF cereals and pseudocereals

GF cereals that are of relevance for beer production are rice, maize, sorghum, millet, and their derivatives. GF pseudocereals that have been used for brewing are amaranth, buckwheat, and quinoa. The main advantage of alternative raw materials is that they are by definition completely devoid of gluten. Besides, GF cereals and pseudocereals possess antioxidative potential, such as via rutin from buckwheat and tocopherol from quinoa, and anti-carcinogenic activities, such as via bioactive polysaccharides from quinoa (CQP) (Holasova et al., 2002; Hu et al., 2017; Tang et al., 2015). Furthermore, they exhibit an antihypertensive effect, induced by ACEi peptides from amaranth, and anti-diabetic properties, for example, via DPP-IV inhibitors from maize and rice (De la Rosa et al., 2010; Lacroix & Li-Chan, 2016). These health-promoting effects were also partly observed from barley, wheat, and rye as well (Adom, Sorrells, & Liu, 2003; Idehen, Tang, & Sang, 2017). A disadvantage is that the malting facility has to be adapted, for example, due to varying grain size, with maize having the highest thousand kernel weight and amaranth the lowest. Second, malting parameters differ from those of barley, with a potential need for higher (millet, rice, sorghum) or lower temperatures (amaranth, quinoa), prolonged germination times (rice, maize), and increased steeping regime (amaranth, quinoa, millet) (Zarnkow, Keβler, Burberg, Kreisz, & Back, 2005). This increases the risk of mold formation and enhanced rootlet growth, which results in malting loss. In addition, the handling of some GF malts is difficult due to higher friability of the grain (buckwheat). In comparison to barley, the compositions of proteolytic, cytolytic, and especially amylolytic properties differ greatly. While barley carbohydrates account for 62.7%, concentrations in millet (68.2%), maize (65%), and especially rice (73.7%) are increased (Gobbetti & Gänzle, 2013). The total carbohydrates in pseudocereals are lower on average than in barley (Eggum, Kreft, & Javornik, 1980; Nowak, Du, & Charrondiere, 2016). Although the higher carbohydrate concentrations of GF cereals seem to be advantageous for beer production, a disadvantage is that most of these grains have a higher gelatinization temperature (see Table 1). This is mainly due to the modified properties of amylopectin, such as entanglement and chain length (Lin et al., 2013), which results in insufficient saccharification if no process modification is adopted. Another problem with alternative grains is their low enzymatic capacity (Taylor, Dlamini, & Kruger, 2013), whereas especially α- and β-amylase activities are lower in maize, rice, and sorghum in comparison to barley (see Table 1). The same results were obtained from amaranth, buckwheat, and quinoa (Zarnkow et al., 2005). This lack of activity can possibly partly be substituted for by the contribution of other amylases such as α-glucosidase and limit-dextrinase, as shown in rice and sorghum (Taylor et al., 2013). To compensate for these disadvantages, the brewhouse facilities and procedures have to be modified by applying an adjunct cooker to compensate for the gelatinization temperature, separate mashing in, and pH adjustment of the mash to preserve enzymatic activity. Alternative, pretreated cereal extracts such as hydrolyzed starch or liquid sugar surrogates added during wort boiling or the addition of exogenous enzymes are also applied. Nonetheless, resulting worts can obtain high viscosities of 2.0–13.3 mPa × s with amaranth, buckwheat, maize, and sorghum, pH of 6.2 with amaranth, and a darker color of 13–14 EBC with quinoa, millet, and sorghum (Zarnkow et al., 2005). Although secondary plant components such as higher zinc values (quinoa), higher amounts of fatty acids (millet), and higher amounts of free amino nitrogen (buckwheat) may provide substrates for enhanced yeast metabolism (De Meo et al., 2011; Zarnkow, Geyer, et al., 2007; Zarnkow, Keβler, et al., 2007; Zarnkow et al., 2009), the resulting beers differ in apparent attenuation limit in the range of 10.7%–60.3% (amaranth, buckwheat, maize, sorghum), whereas they contain lower levels of alcohol of 0.6%–3.75% (v/v) (Zarnkow et al., 2005). Owing to altered carbohydrate and protein composition, the aroma of GF beer made from GF grains is different to that of barley, which can provide a negative sensation (Dezelak, Zarnkow, Becker, & Kosir, 2014). Additionally, owing to higher lipid content and lower total nitrogen, foam stability is very low when using millet,