

Review

Gluten-Free Brewing: Issues and Perspectives

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Abstract: Celiac disease (CD) is an immune-mediated gluten-sensitive enteropathy. Currently, it affects around 1% of world population, but it is constantly growing. Celiac patients have to follow a strict gluten-free (GF) diet. Beer is one of the most consumed beverages worldwide, but it is not safe for people with CD. It has a gluten content usually above the safe threshold (20 ppm), determined by the official method for hydrolyzed foods (R5-competitive-ELISA). The demand on the market for GF beers is increasingly growing. This review aims to provide a comprehensive overview of different strategies to produce GF beer, highlighting strengths and weaknesses of each approach and taking into account technological and sensory issues. GF cereals or pseudocereals have poor brewing attitudes (if used as main raw material) and give the beer unusual flavour. Instead, enzymatic treatments allow traditional brewing process followed by gluten content reduction. A survey on 185 GF-producing breweries (both industrial and craft) from all over the world have been considered to assess which approach is most used. Beers brewed with GF cereals and pseudocereals (used in well-balanced proportions) are more common than gluten-removed (GR) beers, obtained by enzymatic treatment.

Keywords: gluten-free; beer; brewing process; cereals; pseudocereals; sensory analysis

1. Introduction

Beer is one of the most ancient and consumed beverages worldwide [1]. It is a fermented alcoholic beverage made from malted cereal grains, water, hops and yeast. The definition of beer is not the same for all countries: each gives its indications regarding raw materials and the method of production. According to Reinheitsgebot, the beer purity law introduced in Germany in 1516, the main ingredients for the beer production were water, malt and hop. Yeast was considered as a necessary ingredient for beer only later [2], but specific starter cultures are included in the product specification of traditional beers such as ‘Münchener Bier’ [3]. Global beer market is growing in the last years: it is forecast to reach more than \$ 600.000 million by 2025 [4]. Currently, China is the main producer of beer in the world, it produces approximately 381 million hectoliters per year, followed by USA and Brazil. Germany holds the record of consumption and production in Europe [5]. Beer market is segmented based on type of beer, by category, packaging, region or by production. Segments of beer market, growing in the recent years, are those concerning: 1) non-alcoholic beers (divided into alcohol-free and 0.5% alcohol by volume), of interest to the consumer due to an increasing number of heart diseases, strict laws on alcohol consumption and a greater attention to a healthier life [6]; 2) organic beers, made only with organic ingredients and free from GMO, since the consumer is more interested in avoiding undesired chemicals and in protecting environment [7]; 3) craft beers, processed by “small”, “independent” and “traditional” breweries, because the consumer is increasingly attracted by premiumization aspect,

artisanal products and by a well-detailed aromatic profile [8] and 4) gluten-free (GF) beers, brewed with GF cereals or processed to remove gluten, safe for people with celiac disease (CD) but also drunk by consumer who follow eating habits [9]. It is just about the last type of beer that this review wants to consider. Common beer is not allowed in the GF diet. It is brewed especially with barley and wheat, which are gluten-containing cereals. Therefore, it is not safe for people with CD because it contains a gluten content above the safe threshold established by European Legislation for “GF” status. The safe threshold was established by performing several trials. Catassi et al. [10] conducted a prospective, double-blind, placebo-controlled trial to establish this threshold. Forty-nine celiacs ingested for 90 days a capsule containing 0, 10 or 50 mg of gluten. Mucosal negative changes emerged in some patients, especially with a gluten intake of 50 mg/d in the diet, whereas 10 mg/d did not show adverse effects. Thus, the value of 20 ppm was chosen in a preventive way as safe threshold, in order to avoid adverse effects caused by a high ingestion of gluten [11]. Although during the brewing process the gluten level naturally decreases, it remains above 20 ppm in the most of cases. Gluten content of craft beers may be higher than conventional ones, as pointed out also by Fanari et al. [12], because protein component is not removed by filtration (not allowed in the microbrew according to Italian legislation [13]). Several authors [12,14–16] determined the gluten content in different styles of beers, by RIDASCREEN Gliadin competitive assay. Most of them were considered “low of gluten” (<100 ppm of gluten), for example, Stout, Pilsner, Abbey/Trappist, Amber, Pale Ale, unlike wheat beer that had higher gluten content compared to all-barley malt beer. Only in a few cases gluten content was below 20 ppm, as in the case of some Pilsner or alcohol-free beers. However, this may depend on the recipe followed in the brewery. Currently, brewers seek more information about strategies to produce GF beer and to satisfy consumer’s requirements. Therefore, new technologies of brewing are developed, but the final product has different sensory attributes and a higher cost than conventional beer [17]. This review aimed to describe the different approaches to produce GF beer. The use of GF cereals as raw materials and enzymatic treatments are the main strategies used to reduce gluten content in beer. Technological and sensory aspects are also considered for each approach. In addition, a representative list of industrial and craft GF breweries available on the global market has been proposed, distinguishing them according to which approach has been used for the production.

2. Gluten-Related Disorders

Proteins are divided in albumins, globulins, prolamins and glutelins, according to their solubility in different solvents. Prolamins and glutelins contain high glutamic acid and proline content. Prolamins are defined “storage proteins” [18] and they have different names, according to the cereal from which they derived: in wheat they are called gliadins, zeins in corn, kafirines in sorghum, hordeins in barley, avenins in oat and secalines in rye [19]. Prolamins in wheat, barley and rye are toxic for people with celiac disease, because they are immunoreactive with celiac T-cells. On the other hand, rice, sorghum and corn prolamins lack of epitopes that cause allergic reactions. Additionally, millet, teff and pseudocereals (chia, quinoa, buckwheat and amaranth) are considered safe for celiacs [20]. Gluten is defined as “a protein fraction from wheat, rye, barley, oat or their crossbreed varieties and derivatives thereof, to which some people are intolerant and that is insoluble in water and 0.5 M NaCl” [21]. Gluten ingestion may cause different disorders, with different pathogenesis and a different immune response. They are classified in autoimmune (celiac disease, gluten ataxia and dermatitis herpetiformis), allergic (wheat allergy) disorders or unknown mechanism disorders (gluten sensitivity) [20]. Each type of disorder is related to specific amino acid epitopes [20]. Symptoms of gluten-related disorders are abdominal cramps, diarrhea, gas, nausea, iron deficiency anemia and, gastroesophageal reflux [22]. Celiac disease is one of the best-known gluten-related disorders, but not the most widespread. In Italy, about 1% of population is affected by CD [23]. Immunoreactive sequences related to CD are α -gliadin 57–73, Gli2 57–89, also known as “toxic 33mer”, ω -gliadin/C-hordein and B-hordein [20]. Genetic tests are performed to verify the presence of HLA DQ2/8 alleles, because their presence is the genetic factor triggering the disease [24], but also serologic testing with the use of IgA class human anti-tissue

transglutaminase (anti-tTG) antibody are used to screen for CD. However, results obtained from these tests require confirmation by intestinal biopsy [22]. The therapy against gluten-related disorders is to follow a GF diet, excluding foods that contain wheat, rye, barley and oat (if it is produced in conventional production chain with potential contamination with cereals mentioned above) and their crossbreed varieties, or other products used as an ingredient, such as malt extract, wheat starch, bran or germ. Their presence in foods must be reported on the label even if the term “gluten” is not expressly mentioned [22]. People with CD, who exclude cereals containing gluten from their diet, have some nutritional deficiencies, for example, vitamin and fiber weaknesses, reduced level of iron, folate, zinc and magnesium [25]. These macro- and micro-nutrients must be derived from increased intake of legumes, GF cereals or pseudocereals, fruits and vegetables, in order to avoid micronutrient deficiencies and an unbalanced diet [26]. Alternatives to GF diet can be pharmacologic therapies. There are, for example, some oral enzyme supplements, i.e., proteinases from germinated barley seed (EP-B2), from *Sphingomonas capsulate* (SC-PEP), prolyl-endopeptidases deriving from *Aspergillus niger* (AN-PEP), polymeric binders, each of them able to eliminate the immunotoxic effect of gluten peptides. Their high cost is an obstacle to the development of these alternative therapies [27].

3. Methods for the Determination of Gluten

Determination of gluten in food is essential to guarantee the safety to people with CD. The official method for its determination is described in Codex Alimentarius standard for foods for special dietary use for people intolerant to gluten [21]. The official method for determination of gluten is an enzyme-linked immunosorbent assay (ELISA) based on the Mendez method. Throughout this method, toxic peptides present in the structure of gluten are recognized by R5 monoclonal antibody [21]. However, this technique has some limitations, especially for the determination of gluten in hydrolyzed foods, such as syrups, malt extract, breakfast cereals and beer, in which gluten content may be underestimated [28]. A competitive assay is more appropriate for the detection of gluten in fermented products or partially hydrolyzed peptides of gluten, such as in beer, syrups or sourdough. In these cases, the official method is R5-competitive-ELISA [29]. Alternative techniques for the determination of gluten are, for example, liquid chromatography tandem mass spectrometry (LC–MS/MS), very selective towards many analytes and it detects several allergens simultaneously, too [30,31]. Mass spectrometry is highly sensitive, can directly detect proteins/peptides not detected by immunological techniques, but it requires expensive equipment and expertise [32]. In a study conducted by Colgrave et al. [33], different beers (barley-based and gluten-removed beer) were analyzed by LC–MS/MS to estimate the gluten content and to reveal hydrolyzed peptides, too. A higher gluten-derived number of peptides was detected by LC–MS/MS in low gluten beer, treated by prolyl-endopeptidases (PEP) to remove gluten, than the ELISA method. Additionally, Tanner et al. [34] analyzed sixty beers and compared ELISA results with multiple reaction monitoring–mass spectrometry (MRM–MS) ones. Some beers proved near-zero gluten level by ELISA, whereas a higher presence of hordein was detected in the same beer analyzed by MS. Besides, DNA-based methods, such as polymerase chain reaction (PCR), was used to detect wheat, barley and rye contamination. DNA is more efficiently extracted compared to proteins and is a stable analyte, too. Some samples, analyzed by Mujico et al. [28], resulted negative with ELISA, but slightly positive with quantitative real-time PCR (Q-PCR) system. However, these methods may be unsuitable for highly processed or fermented-hydrolyzed foods [32]. Given the above, it is essential to select the best method for the determination of gluten based on the characteristics of the matrix considered.

4. Legislation and Market of GF Foodstuffs and Beer

According to the Association of European Coeliac Societies (AOECS) standard for GF foods, a food product is defined “gluten-free” if it does not contain cereals such as wheat, rye, barley, oat or their crossbreeds hybrids, for example, triticale. If it contains these ingredients, it has to be processed to remove gluten, in order to remain under the level of 20 mg/kg [29]. In this way, it can receive

the crossed grain symbol, a quick and easy way of identifying the foods for people with CD [35]. The difference between “gluten-free” and “very low gluten” statement is specified in the European Regulation on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. In the first case, the gluten content is less than 20 ppm, for the latter the threshold is considered <100 ppm [36]. Instead of the European Regulation, the “processed, treated or crafted to remove gluten” claim is used in America [37], whereas in Australia and New Zealand a “no detectable gluten” standard is applied [38]. The GF market is expected to grow in the next years. From 2020 to 2027 it will grow with a compound annual growth rate (CAGR) of 9.2%, due to increasing incidence of celiac disease, but also for more attention in preventing health disorders and in following healthy lifestyles. Currently, due to new technologies, there are different GF products on the market. The innovative process makes them also more palatable [39], focusing on improvement of nutritional and sensory aspects of GF products [40]. GF beer market is one of the segments of GF products trade that is growing in these years. It is estimated to reach USD 651.6 million with a CAGR of 14.90% in the forecast period (2018–2023) [9]. Another driver of this increment is the easier availability of GF beers on the market, due to an increase in the number of breweries interested in this kind of production. Moreover, the consumer wants to try new products and this has led the beer companies to expand their portfolio. Nevertheless, higher costs of GF beer than conventional ones are the main limitation of the GF beer market [41]. AOECS has licensed over 20,000 GF products to use the Crossed Grain Trademark (CGT), if they contain less than 20 ppm of gluten. In this review, the case study of the world of Italian GF beers has been reported. In Italy people with CD may consult the list of crossed symbol grain products made by Associazione Italiana Celiachia (AIC) in order to choose packaged food products, which are safe and suitable for the specific requirements for the GF diet. Six Italian GF beers are included in this list: Amo Essere Senza Glutine-Best Brau (Eurospin Italia), BeneSì Senza Glutine (Coop), Birra Premium Senza Glutine (Birra Alpen), Igea (Birra Salento), Birra Superior Senza Glutine (Fabbrica di Pedavena) and Peroni Senza Glutine (Peroni) [42]. According to the Italian legislation for conventional beer [43], barley and/or wheat cannot be replaced for more than 40% of the dry wort extract by other cereals or starchy and sugary substances. Therefore, if only GF grains are used as raw material, the final product cannot be defined as “beer”, but as “alcoholic beverage based on”. Most of the 46 Italian GF beers reviewed on the web are craft [44]. Gluten content below 20 ppm is reached or by substituting less than 40% of barley with other ingredients, for example corn, buckwheat or quinoa, or taking into account technological devices. Two of these ones must not be considered “beer” because they are produced by 100% of rice malt and quinoa malt, respectively. This restriction does not apply in most other countries around the world. Sometimes, the minimum malt content is not reported: it does not matter what raw materials are used and in what concentrations, as long as the GF statement is respected [45]. In order to characterize GF beers available on global market, a list of 185 breweries reviewed on the web has been analyzed [46,47]. They derived from 24 different countries around the world. Breweries of each nation have been divided according to production method: gluten-free (GF) refers to breweries, which use GF ingredients (respecting the concentration indicating by reference legislation) or reach the threshold simply by technological devices, whereas gluten-removed (GR) refers to breweries that use conventional raw materials, such as barley malt, and then remove gluten by applying enzymatic treatment. The majority of breweries are located in USA, followed by Italy and England. American breweries produce mainly by the GF method, unlike in England where the enzymatic treatment is predominant. Bearing in mind the global trend, 62.16% of breweries produce by the GF method, whereas 37.84% by the GR treatment. Table 1 shows the distribution of these breweries around the world and what method they use.

Table 1. Distribution of breweries according to production method: gluten-free (GF) or gluten-removed (GR).

Country	GF	GR
USA	33	10
Italy	33	nd
England	3	27
Spain	3	7
France	6	2
Belgium	6	2
Germany	6	2
Canada	7	nd
Denmark	nd	4
Scotland	nd	4
Australia	3	nd
Mexico	2	1
Argentina	3	nd
Austria	2	1
Ireland	1	2
New Zealand	3	nd
Chile	2	nd
Poland	nd	2
Finland	1	1
Czech Republic	nd	2
Israel	1	nd
Brazil	nd	1
Norway	nd	1
Holland	nd	1

nd = no data.

5. Approaches for GF Beer Production

Gluten content decreases during germination due to a modification of grains structure, but also during wort separation, cooking, fermentation due to the pH reduction and the development of proteins-polyphenols complexes, with wort and beer clarification to prevent haze formation. However, a small percentage of gluten remains in the final beer. This is why, in the last years, different approaches have been used in order to bring the gluten content below the GF threshold. The main approaches for GF beer production used so far are: the use of GF cereals or pseudocereals, no-grain materials, enzymatic and precipitation treatments. Innovative approaches are developed in the recent years, too. Among these different approaches, it should be taken into account that only GF beers, obtained by grains that are naturally free from gluten, are definitely safe for celiacs, because there is no trace of gluten from the beginning to the end of the brewing process. Besides, GR beers may not be safe for celiacs because, after the reduction treatment, some protein fragments may remain in the final product, triggering an allergic response. Therefore, GR beers are suggested in case of gluten sensitivity, but further studies are needed to validate the effectiveness of the reduction treatment and hence the consumption of GR beers by people with CD [48].

5.1. Use of GF Cereals or Pseudocereals

The main cereals used in GF industry are rice and maize [40], but there are other raw materials that provide interesting nutritional and sensory properties. Of course, each of these confers different technological properties, because of a different content and structure of starch and hence different temperature of gelatinization, also due to a different botanical origin [49]. As demonstrated by Yu et al. [50], starch molecular structure and content, amylose and amylopectin ratio, enzymatic activity, temperature of gelatinization and hence fermentable sugar content affect performance of brewing. GF cereals and pseudocereals have temperature of gelatinization above the optimal temperature of amylolytic enzymes, resulting in lower fermentable sugar content and more non-fermentable sugar

(the latter are not used for fermentation process). However, the most common cereals used to obtain GF beer are oat (if produced under a GF purity protocol), rice, maize, sorghum, teff, millet, buckwheat, quinoa and amaranth. Rice and maize are already used in conventional beer production, because brewers often use them as adjunct, in order to reduce the price of final product, since these cereals are cheaper than barley [51], unlike the other raw materials mentioned above. In the following paragraph, the use of each GF cereals or pseudocereals in brewing process is reported, exploring their technological and sensory issues. Table 2 reports brewing attitudes of GF cereals and pseudocereals.

Table 2. Brewing attitudes of gluten-free (GF) cereals and pseudocereals.

Grain	Gelatinization Temperature (°C)	Saccharification Time (min)	Diastatic Power (WK units)	Kolbach Index (%dm)	Extract of Malt (%dm)	Alcohol ^a (%)	Foam Stability ^a (sec)	References
Barley	58–66	10–15	200–416	34–45	76–88	3.65–5.32	228–263	[52–56]
Oat	52.6–62	15	82–124	30–37	62.1	4.58–5.25	150	[52,53,57,58]
Rice	67–91	>30	19–62	15.1–39.3	64.3–77.8	3.63–5.12	157–182	[58–64]
Maize	62–80	55	77	32.6–33.2	68–68.75	3.60	75.6	[58,64–68]
Sorghum	69–80	60	72–101	20–26	68	2.9–4.66	130	[55,56,64,69–71]
Teff	69–80	60	87	21–33.4	74.2–82.3	4.37–4.68	154–181	[58,72–75]
Millet	54–80	35	40–61	39.5	59.6–68.9	2.55–4.7	data not reported	[54–56,64]
Buckwheat	65.4–72	>20	72	28–32.3	61.9–65.3	3.79–4.7	data not reported	[58,76–79]
Quinoa	64	>20	61	53.4	37.7	4.3–4.9	319	[58,64,76,80]
Amaranth	64–74	>60	data not reported	15–20	88.6–91.1	4.6	data not reported	[64,75,81,82]

WK = Windisch–Kolbach; dm = dry matter; ^a (referred to the final product).

5.1.1. Oat

Oat (*Avena sativa*) is an annual grass belonging to the family of Poaceae produced mainly in Russia, Canada and USA. Oat is considered a functional cereal: it is a good source of antioxidants, such as vitamin E, phytic acid and phenols [83]. There are different opinions for the use of oat in the GF diet. It can be used in products labeled “gluten-free” only if it is produced, prepared or processed in a way to avoid contaminations by wheat, rye, barley or their crossbreeds and it must contain a gluten content < 20 ppm, too [84]. Oat is used in brewing process. This cereal has a lower diastatic power (DP) and a lower gelatinization temperature than barley. Therefore, brewing with 100% malted oat results in low extract content, a high pH value and low alcohol content. Oat wort has a high viscosity causing problems during beer filtering because of its high content of β -glucans and proteins. Oat malt has quite low free amino nitrogen (FAN) and total soluble nitrogen (TSN) content as a result of partial proteolytic modification of malt. α -amylase activity is lower than barley. Nevertheless, also taking into account the sensory characteristics, beverage from 100% oat malt is comparable with that obtained by a barley-based malt [52]. However, due to a high amount of unsaturated fatty acids, oat beverage is more susceptible to the development of off-flavors and rancidity [53]. The impact of various levels of unmalted oats (0%, 10%, 20%, 30% and 40%) on the quality of mashes, worts and beers was evaluated. β -glucans content and viscosity of mashes increased significantly with increasing amount of unmalted oat [85]. In another study the addition of commercial exogenous enzymes improved the quality of beer with oat adjunct, achieving a higher extract content and FAN level, a higher volume of wort, a lower viscosity and hence a faster wort filtration. Therefore, the use of enzymes was recommended when more than 22.5% of oat is added to wort [53]. In conclusion, the use of 100% of oat malt is not recommended, because it leads to low extract content due to a low DP and thus low alcohol content, and also it causes filtration problems due to high β -glucans levels. Thus, oat is more efficient to use as adjunct, for example, in the form of unmalted oat, and adding it directly into the mash.

5.1.2. Rice

Rice (*Oryza sativa*) is one of the most consumed staple foods in the world, especially in Asia. It belongs to the Poaceae family. Due to its high starch content (about 70%–75%), rice is a suitable raw material for brewing, even if it has a low nitrogen content, necessary for yeast nutrition [62]. In the beer process, it is generally used as an adjunct because it can significantly increase the extract content of a mash [60,61]. The different structure and composition between rice and barley entails the need to optimize malting and mashing conditions. In fact, rice malt has low DP and high gelatinization temperature. As reported by Mayer et al. [59], the ideal temperatures for enzymes in rice malt are 65 °C for limit-dextrinase and β -amylase and 74 °C for α -amylase. Rice, unlike other cereals, germinates under anaerobic conditions in order to activate α -amylase II-4 isoform [86]. As pointed out by Usansa et al. [86], the best malting conditions occurred with a steeping step at 30 °C for 24 h and germination phase for 4–5 days, resulting in a suitable α and β -amylase activity. Despite a lower DP than barley, rice can be a good raw material for brewing because it has a higher limit-dextrinase content than barley that allows the complete saccharification [61,87]. The different amyolytic activity between rice and barley was showed also by Mayer et al. [59], observing the different sugar profile: maltose and glucose were in the same range in rice, and it contained a higher maltotetraose, maltopentaose and maltohexaose content than barley. Due to its high unsaturated free fatty acids content, rice is more susceptible to oxidation, with the risk of rancid odor development. The positive aspect linked to use rice in brewing is its good fiber content, which facilitates the lautering process due to its filtering capacity [88]. Although rice has higher starch content than barley, it has a different starch structure, different composition in amylose and amylopectin and lower amyolytic activity than barley. Therefore, using rice in the brewing process leads to more soluble starch in the wort, not used by the yeast. This results in a lower amount of ethanol production, also due to a lower soluble protein content [50]. For all these reasons, it would be appropriate to use rice as adjunct instead of rice malt, due to a lower production costs, but also because, using only rice as raw material, final product would have too flat sensory characteristics [59].

5.1.3. Maize

Maize (*Zea mays*), also known as corn, belonging to the Poaceae family, is widely cultivated, second only to wheat in total production area and second to rice in amount produced [89]. It is used as adjunct in the brewing process to improve wort and beer quality because it is a good source of carbohydrates useful for the yeast. Besides, the prize of the entire production process should decrease with its addition because it is a cheap raw material [90]. About maize brewing attitudes, it has a high starch gelatinization temperature, α -amylase activity several times higher than sorghum but lower than rice malt, slightly low β -amylase activity. The major endosperm cell wall components in maize are arabinoxylans, unlike in barley, which persist during malting [91]. Poreda et al. [90] produced a beer with the addition of 10% and 20% of corn grist in order to analyze their influence on the brewing process and beer quality. Both corn adjuncts influenced wort color: the intensity decreased (11.1 °EBC with 10% corn grist adjunct and 10.5 °EBC with 20% of corn grist). Dimethyl sulphide (DMS) and non-fermentable extract were significantly low in the wort produced with adjunct. FAN content also decreased because, with corn adjunct, hardly hydrolysable protein content increased. Final product characteristics were not significantly influenced. In addition, as stated by Diakabana et al. [65], maize beer has a low alcohol content, normal pH (about 4.5) and brown color due to the Maillard reaction. The future trend to stimulate the use of corn in brewing is the use of exogenous enzymes to improve saccharification, or genetic modification to help amylolytic activity [91]. So, in conclusion, the best way to use maize in brewing is certainly as adjunct (corn grits are the most common form), acting as a source of starch or sugar. Moreover, malting process of maize is difficult and expensive and, in fact, not very common in brewing.

5.1.4. Sorghum

Sorghum (*Sorghum vulgare*), belonging to the Poaceae family, is one of the major food sources of energy and protein in African countries. There are some problems in brewing with sorghum malt due to low diastatic activity, insufficient for a complete saccharification, high gelatinization temperature but also low FAN content [91]. Sorghum has a low β -amylase activity, but a higher α -amylase activity than barley malt. This leads to low fermentable sugars production and a high dextrins content, causing an increase of viscosity [92]. Gelatinization temperature is limited by kafirins. Therefore, starch hydrolysis into fermentable sugars occurs only partially [93]. For these reasons, the use of sorghum in brewing requires the development of an appropriate malting process; otherwise, exogenous enzymes may be used to produce sorghum beers, avoiding technological issues. In this regard, Espinosa-Ramírez et al. [94] evaluated the effect of β -amylase or amyloglucosidase addition during mashing of sorghum, achieving a higher alcohol content. Amyloglucosidase (AMG) was added also by Urias-Lugo and Salvidar [95], resulting in an improved wort yield and filtration rate and a higher % of ethanol. However, alcohol content of sorghum beer contained approximately 1.1% less than barley malt beer. Color, pH and FAN content were not affected by AMG adjunct. *Aspergillus oryzae* was also added to enhance the brewing properties of sorghum and to eliminate the gap with barley malt. α -amylase was positively affected by this adjunct, whereas for β -amylase there were no differences [93]. The use of sorghum as the main raw material in brewing is traditional in Africa, both as malted sorghum and as adjunct [96,97]. In addition, sorghum beers are already on the market, which means that it lends itself well to brewing. Nevertheless, it is necessary to improve malting conditions and the final sensory aspects (maybe using it in combination with other cereals).

5.1.5. Teff

Teff (*Eragrostis tef*) is a tropical cereal, belonging to the family of Poaceae, deriving from Ethiopia. Teff has a high nutritional value and functional properties, rich in starch (about 73%), protein (11%–12%), with a relevant content of minerals, phytic acid, polyphenols, dietary fiber and essential amino acids, making it interesting in food applications and for the celiac diet [58,74]. Di Ghionno et al. [74] evaluated

the behavior of a particular variety of teff during the brewing process. Lipid content resulted lower than barley, but this was useful to prevent collapse of foam beer and off-flavor caused by oxidation in aging beer. pH of teff wort was higher than barley (6.0–6.2). Therefore, it could adversely affect the enzymatic activity. Malted teff had a low DP, but it did not influence the teff attitude for brewing because other amylolytic enzymes, such as limit dextrinase, pullulanase and α -glucosidase, contributed to the degradation of starch and the production of oligosaccharides, consequently used by α and β -amylase. The different enzymatic activity compared to barley led to a different sugars profile: teff malt had more glucose than maltose. A negative aspect of teff malt was the high gelatinization temperature due to the small size of starch granules with high crystallinity and structural differences in amylose and amylopectin [73]. One hundred percent teff malt was compared with 100% raw teff supplemented with exogenous hydrolytic enzymes, such as amylase, β -glucanase, xylanase, proteinase and pullulanase. Extract content was similar in both cases. There were no differences in fermentability attitude, even if it was lower than in barley wort. Raw teff wort had a TSN content significantly lower than malted teff, probably because teff proteins were not degraded by exogenous enzymes. FAN content of malted teff resulted in a higher apparent limit attenuation. In summary, the main differences between raw and malted teff were in FAN, TSN content and free amino acids composition due to different action of enzymes produced during the malting process [74]. Usually, teff is used as unmalted adjunct, also because it is one of the smallest grains in the world and so it is difficulty in managing. However, its use in a recipe, even if in small concentrations, is to be considered.

5.1.6. Millet

Millet (*Panicum miliaceum*) is a tropical cereal, derived from Africa, belonging to the *Poaceae* family. Millet is used as a substitute of barley and sorghum malt to produce traditional African beer, called opaque beer. The main technological problems for using millet in brewing are the low DP and the need to optimize the malting process [98]. Millet has high β -amylase activity. That is why it produces a higher maltose concentration than sorghum, a positive factor for the fermentation [98]. Nonetheless, millet malt has a low extract yield, dark color and underdeveloped flavor compared to barley malt. Therefore, millet is better used for opaque beer production, different from the conventional one. A comparative research of experimental beers brewed from millet, sorghum and barley was reported by Agu [55]. Extract yield ($^{\circ}$ P) and attenuation limit (expressed in %) of millet were lower than barley, due to the small size of endosperm available for extract production. Therefore, alcohol content of millet beverage resulted quite low. For this purpose, *Brettanomyces bruxellensis* was used to ferment millet malt in order to get a higher alcohol content and the right choice of strain for fermentation allowed the development of aromatic compounds comparable to those found in barley-based beer [99]. As stated by Nout and Davies [56], a technological disadvantage of millet is its very small grain sizes. This affects the steeping step, because grain absorbs less water, with an impact on DP. Besides, millet contains nutritional inhibitors, as phytic acid, tannins and cyanides. Alkaline steeping can reduce these antinutritional factors [100]. As sorghum, millet beers are already produced in Africa, being these grains traditional in this continent. Even if it has different brewing attitudes than barley, malted millet can be used, but taking into account a very different sensory quality of final product. The use of other grains in combination of malted millet is suggested in order to compensate for this disadvantage.

5.1.7. Buckwheat

Buckwheat (*Fagopyrum esculentum*), belonging to the Poliganaceae family, is considered a “pseudocereal”, as well as quinoa and amaranth. It provides benefits for health, because it contains a high antioxidants content (rutin level, a flavonoid glycoside), a large amount of minerals, polyunsaturated fatty acids, high fiber levels and high biological value proteins. It is also a good source of starch, and so it is used as raw material for bread or pasta production, but also for brewing [78,101]. It may be a good substitute for barley providing reasonable sensory characteristics in the final product. However, there are some technological issues to be aware of: low amylolytic activity and extract yield, low rates

of lautering due to high wort viscosity and fermentation problems [102]. To overcome these drawbacks, optimization of malting process and also exogenous enzymes can be used to compensate the low enzymatic activity and to promote the complete saccharification. Wijngaard et al. [78] conducted different malting trials selecting four different germination temperature with the aim to check which of them gave the better quality malt. Germination at 15 °C for 4–5 days provided reasonable results. Maximum α -amylase activity occurred between second and third day of germination. Then, it leveled off. Instead, the maximum β -amylase activity was reached after five days of germination. As stated by Agu et al. [101], glucose content in buckwheat malt is 3–5 times higher than maltose. This may influence the yeast metabolism because, in malts where glucose level is in excess, yeast may lose its ability to ferment maltose. Buckwheat was also used as adjunct in concentration of 20% and 40%. With 40% of buckwheat the malt viscosity increased compared to 20%, but FAN content decreased [103]. The main disadvantage for brewing with buckwheat is the long saccharification time, leading to partial starch degradation. Therefore, there is the need to use supplementary enzyme [76]. Optimizing the right malting procedure, buckwheat malt can be used as main raw material in brewing, achieving an increased amyolytic activity. However, it is always advisable to use buckwheat in combination with other malts or adding enzymes to improve the enzymatic activity of buckwheat malt.

5.1.8. Quinoa

Quinoa (*Chenopodium quinoa*), belonging to the Chenopodiaceae family, is one of the most important food staples in South America. It is considered a functional food due to its important total phenolic content, great antioxidant activity; it contains also a significant minerals level and vitamin. Quinoa is used in the brewing process even if, just like the others pseudocereals, has low enzymatic activity and different sensory quality than barley malt [80]. Brewing attributes of quinoa are: low malt extract yield, long saccharification time and dark color of final beverage due to high pigment content. Despite this, as mentioned above, quinoa has a high nutritional value, it also produces a low % of alcohol and hence quinoa beverages can be consumed more frequently [76,80]. Quinoa flakes in concentration of 10%, 20%, 30%, and 40% and quinoa seeds at 10%, 20%, and 30% were also used as adjunct in the brewing process. Extract yield decreased with increasing in unmalted raw materials content, due to their low enzymatic pool. Saccharification time increased with 30% of quinoa seeds or 40% quinoa flakes adjunct. With quinoa adjunct, viscosity increased because of a major content of β -glucans leading to filtration problems. As in buckwheat, glucose level was higher than maltose, creating a possible negative effect on yeast fermentation performance [80]. As for buckwheat, when alternative malted grains, for example quinoa, are used by themselves in brewing, the addition of enzymes or source of sugar is required for the success of the process.

5.1.9. Amaranth

Amaranth (*Amaranthus* L.) is a “pseudocereal”, belonging to the Amaranthaceae family. It contains high amount of protein (about 15%) with a high biological value, about 7% of fat and 63% of waxy starch (with a lower amylose content and smaller size than wheat starch), high fiber content, important micronutrients levels that promote fermentation performance and great antioxidant activity [104,105]. To date, few studies on amaranth brewing are available. It has a high temperature of gelatinization and the saccharification is not complete [64]. Brewing with amaranth malt gives a low volume of final beer and also a medium alcohol content in the final product [82,89]. The development of the malting process is still necessary in order to use this functional grain in brewing. However, using it as adjunct can be a novelty in beer market arousing interest from the consumer. Therefore, this could be a challenge for the future.

5.2. No-Grain Materials

Another approach is to use no-grain materials, in order to produce so-called “ersatz” beer, because some ingredients are used as a substitute for something else. Klisch [106] created a way to form an aqueous

brew using water with the adjunct of source of fermentable sugars, yeast nutrient, protein coagulant and hops. Sources of fermentable sugars were, for example, natural sugars, such as maltose, glucose, maltotriose, sucrose and fructose, or syrups from GF cereals obtained by enzymes activity (amylases, glucanases, proteases and xylanase, which convert the starch of grains). These enzymatic reactions could produce undesirable acid compounds affecting negatively the taste of final beer. The beer could also contain other ingredients, such as stabilizers, flavoring agents, coloring agents, effervescence generating agents, antioxidants, preservatives and fining agents to enhance the quality of the final product. Before adding syrup, water could be heated over the 50–100 °C extended temperature range, to facilitate the dissolution of syrup. In another patent [107], honey and molasses were used as sources of fermentable sugars for the nutrition of yeast. Bittering agents, obtained from a plurality of hops, were used. Several types of honey could be used in order to create a final product with different characteristics in terms of color, taste, flavor and aroma. If the sugars content of honey varied, also the alcohol content developed could be different. This method was used also in Japan in order to create the so-called “third beer-type” based on fermentable sugars, yeast extract as sources of amino acids, hop materials as flavoring agents, caramel for color and sources of protein from soybeans, peas or corn [16]. The use of fermentable extract supplemented with dextrans may improve mouthfeel of the final product, whereas syrups with high fermentability give the beer more sensation of dryness to the palate [108].

5.3. Enzymatic Treatment

The use of no-containing gluten cereals in beer production negatively affects aroma, taste and other sensory parameters of beer, in addition to having technological issues. There are other methods for gluten reduction, based on the use of specific enzymes, which allow an extensive hydrolysis of proline-rich proteins and prevent beer haze.

5.3.1. Fungal Peptidases

Peptidases are the most used enzymes for gluten removal. They are food-grade and compliant with legislation, stable and active considering temperature, pH value, alcohol content and other components of the foods, easy to integrate into existing industrial production processes, cost-effective, acceptable and beneficial for the final consumers [109]. Prolyl-endopeptidases (PEP) are the most used enzymes by brewers for beer stabilization because they can preferentially cleaving peptides at the carboxyl side of proline residues, and so they prevent formation of proteins-polyphenols complexes. These enzymes are extracted from microbial metabolism, especially from fungi [110]. PEP from *A. niger* (AN), a food-grade microorganism, are considered generally recognized as safe (GRAS), and hence, they are used in the food industry. The optimal conditions for AN-PEP are a pH value of 4.5 and temperature of 50 °C [111]. AN-PEP is completely effective in removing immunogenic gluten epitopes, leading a gluten content below 20 ppm. Enzymatic treatment reduces turbidity by hydrolyzing haze precursors improving beer stability. Beer color is not influenced by enzymatic treatment, unlike ethanol content, real and apparent attenuation (the conversion of sugars into alcohol and carbon dioxide by fermentation process) and extract [112]. AN-PEP is available in the market as Brewers Clarex, for example. A very low amount of this product (about 2.5 g/hL) can reduce gluten content below detectable limits [20,113]. It is generally added at the beginning of fermentation. Nevertheless, Taylor et al. [114] try to apply AN-PEP to the malting process. There was a reduction of hordein content, but gluten level remained still too high for the GF statement. Kang et al. [110] cloned, purified and characterized a prolyl-endopeptidase from *A. oryzae* (AO), in order to demonstrate the applicability of new enzyme in the food industry, with low production costs and no beer losses. Therefore, AO-PEP is used during the fermentation stage to improve beer stability, too.

5.3.2. Bacterial Peptidases

PEP is expressed in different bacteria: *Flavobacterium meningosepticum*, *Sphingomonas capsulate*, *Myxococcus Xanthus*, *Flavobacterium*, *Aeromonas* and *Pseudomonas*. Additionally, lactic acid bacteria

(LAB) have a pool of peptidases able to hydrolyze wheat prolamine and to eliminate toxic epitopes, but no data are reported for their use in brewing [115]. However, tailored solutions can be transferred from the sourdough technology [116], as already done for other cereal-based products such as pasta [117]. PEP from *Sphaerobacter thermophiles* is considered a novel thermostable gluten-specific prolyl-endopeptidase. It is active in pH range 5.0–8.0 and has an optimum temperature of 63 °C. Therefore, its use can reduce gluten below 20 ppm also during the high temperature of mashing in beer process [118].

5.3.3. Transglutaminase

Transglutaminase (TG) enzymes have been discovered in animals, but also in microorganisms. Microbial transglutaminases (mTGs) are heavily used in the food industry to improve texture, elasticity, appearance, persistency, rheology and other quality attributes of food products [119]. They catalyze two classes of reactions: deamidation and transamidation, by altering proteins structure and by changing the immunogenicity of gluten peptides [115]. According to Taylor et al. [120], high doses of mTG (about 92.5 g/hL) can reduce gluten content below 5 ppm. In Germany, a GF beer was produced by this technology [121]. However, in Europe, the addition of transglutaminase must be declared on the label [119]. mTGs adjunct not adversely affects the food products rheology, because they leave intact the primary structure of gluten. However, they are similar to human TG involved in CD pathogenesis; therefore, there is still doubt about their use in GF diet [109].

5.3.4. Peptidases from Germinated Cereals

Additionally, enzymes produced during the malting process of barley can degrade the celiac-active peptides. They are food-grade, natural enzymes with high gluten specificity [122]. Cereals germination and the extraction of the following enzyme are well established techniques by now [115]. So, their application in the food industry is easy and well-acceptable by consumers. Lastly, no genetic engineering is necessary. However, extracted enzymes have not the same activity of purified enzymes, and they could also be damaged from >2% of ethanol content [109]. As suggested in Knorr, Kerpes et al. [123], their adjunct in brewing process must take place after wort boiling and before fermentation. In fact, enzymes from germinated cereals are sensitive to high temperature and ethanol content. The right choice of germination conditions, for example, 8 days at 18 °C with 48% of moisture, improves the peptidase activity of these enzymes. Furthermore, also cysteine proteases (EP-A and the major abundant EP-B) play an important role in the degradation of hordein during germination, unlike the other enzymes present in green barley malt, such as metalloproteases, serine carboxypeptidases, aspartate endoproteases and serine endoproteases [20].

5.4. Precipitation Treatment

Brewers use different strategies for beer clarification and stabilization. The products used with this aim have a proved ability in gluten reduction, too. Application of silica gel or tannins may require subsequent filtration or centrifugation, leading to increased production costs.

5.4.1. Silica Gel

Silica gel is used by brewers as a stabilizer to reduce protein content responsible for haze formation. Proteins involved in foam retention are not affected by stabilizers application because they do not have a high content of proline. Silica gel contains a network of pores, covered with silicon groups (Si-OH), with a great affinity for prolamine residues. Filtration or precipitation are required to remove the flocs forming [112]. Silica gel addition during fermentation maximizes the gluten reduction without changing the yeast performance. However, this compound causes polysaccharides reduction, obstructing filtration, but, compared to enzymatic treatment, it does not obstruct the following stabilization process [124]. Fanari et al. [112] demonstrated the difference between GF beer subject to enzymatic treatment and precipitation treatment with silica gel. Both silica gel and AN-PEP were

added at the beginning of fermentation. Both treatments were able to reduce gluten below 20 ppm, but with some differences in chemical–physical and sensory attributes. There was a gluten reduction of 58% in matured controlled beer compared to green beer, whereas 92% and 63% in enzymatic treated (ETB) and precipitation-treated beer (PTB), respectively. In PTB there was a lower amount of esters, important volatile compounds for beer and fermentation performance was higher in ETB than PTB, because the protease activity increased the amount of nitrogen, required to yeast nutrition.

5.4.2. Tannin Acid

Tannin acid is a mixture of hydrolysable tannins extracted from plants. They are used in brewing as beer stabilizers, because they form an insoluble complex with proteins/polypeptides in a few minutes, subsequently removed by filtration or sedimentation thus providing gluten reduction, too. However, in comparison to silica gel, tannin acid reduces hordein levels, but in a much less effective way. A high dose of tannins (about 20 g/hL) should be used to reduce gluten content below the safe threshold, but this may negatively affect the sensory quality of the final beer, unlike silica gel. In fact, its application at high concentration (about 500 g/hL) reduces gluten content below 4 ppm without affecting sensory characteristics [125].

5.4.3. PVPP

Polyvinylpolypyrrolidone (PVPP) is also used as a beer colloidal stabilizer. It absorbs the haze-active polyphenols. Then, the complexes are removed by filtration. In a previous study, PVPP application, at a concentration of 500 mg/kg, in the beer process has not efficiently reduced gluten concentration [111,124].

5.5. Genetic Engineering and Innovative Approaches

In the last years, genetic engineering has been applied to remove toxic epitopes in gluten-containing cereals, such as wheat or barley. It is a technique still very used, but could not be accepted by national legislation or by consumers [64]. Down-regulation by RNA interference (RNAi) technology reduces the expression of genes correlated to many group of gliadins and/or glutenins, and also reduces the immunotoxic activity of gluten. This technique is used in bread wheat, but no data are currently reported for its application in brewing process [115]. Additionally, plant breeding strategies could be an alternative for the use of gluten-containing cereal grains in the GF diet, for example, for the production of malts, foods and beverages in GF market. Tanner et al. [126] applied the traditional breeding strategy to create an ultra-low gluten (ULG) barley variety, by combining three alleles that together contributed to reduce hordein content. They achieved a grain with successful characteristics for malting and brewing. Gene editing using CRISPR/Cas9 can remove or modify DNA sequences coding for immunogenic peptides. The products derived from this innovative technology may be available in United States, Argentina and Canada, but not in Europe because they are not allowed by legislation [127,128]. The use of hydrodynamic cavitation in wort and finished beer can be considered a novel approach. The researchers used this technique with the aim of preserving taste, aroma and flavor of the beverage without using chemical additives, enzymatic treatments or filtration process. This method is not yet well established and with high costs, nevertheless, it allows the reduction of gluten concentration [129].

6. Sensory Aspects of GF Beers

Beer is a complex matrix and its sensory evaluation is challenging. There are more than 1000 compounds, which interact each other and determine the characteristic aroma and flavor of the final beer. Sensory aspects of final product obtained by using GF cereals and pseudocereals as raw material are summarized in Table 3.

Table 3. Sensory aspects of beers/beverages based on gluten-free (GF) cereals or pseudocereals.

Brewing Materials		Appearance	Aroma	Taste and Mouthfeel	Reference
Oat	40% unmalted oat	Reduction in foam stability	Acceptable aroma	Acceptable purity of taste	[85]
	100% oat malt	Color slightly darker than barley Poor foam stability	Raspberry Blueberry Yoghurt	Creamy Silky Delicate taste	[52]
Rice	100% rice malt	Pale yellow color White foam which rapidly collapses	Flat flavor Malty	Vanillin taste Slightly sour Malty Sweet	[59]
Maize	100% corn malt	Clear Light yellow color Poor foam stability	Cooked maize flavor Sweet corn aroma	Slightly bitter Sweet	[65]
Sorghum	100% sorghum malt	Higher color value Poor foam retention	Estery Fruity	Slightly sour, bitter and astringent taste	[55,130]
Teff	Malted teff	Higher color than raw teff Poor foam stability	Cereal/grainy Worty	Malty Light body Sweet/bitter balanced	[74]
	100% raw teff	Poor foam stability	Banana, pear, apple, tropical fruit as pineapple	Sweet	
Millet	100% millet malt	Dark color Good foam retention	Poor flavor Cidery, bread yeast aroma	Slightly crackery Raw grain taste Cottony sensation in the mouth	[55,56]
Buckwheat	20–40% buckwheat malt	Increase of color	Acceptable odor	Too bitter Acceptable taste and mouthfeel	[103]
	100% buckwheat malt	Opaque Brown color Poor foam stability	Fruity/banana-like aroma Nutty	Good purity of taste	[76,131]
Quinoa	30% quinoa flakes	Darker color	Acceptable aroma	Acceptable taste and mouthfeel	[80]
	100% quinoa malt	Slightly opaque Dark yellow color Grayish foam Quite good foam stability	Banana flavor Malty Alcoholic Nutty aroma	Grainy raw-material Bitter Bad purity of taste Astringency taste Bitter	[76,131]
Amaranth	100% amaranth malt	Slightly opaque Yellow color Poor foam retention	Acceptable aroma but not good freshness flavor	Bad bitterness intensity Full body taste	[89,131]

High fat content in oat wort and beer influences foam stability, as well as the high degree of proteolysis in the oat malt. Both esters and higher alcohols were less than in barley malt. Fruity and yoghurt-like flavor were perceived in fresh oat beer [52]. According to sensory analysis conducted by Schnitzenbaumer et al. [85], oat-containing beers have acceptable sensory attributes. Moreover, oat beer is more susceptible to the development of bitter off-flavors, due to the presence of long-chain hydroxyl fatty acids, and rancidity, associated with volatile aldehydes, ketones and alcohols [53]. Rice beer generally has a low alcohol content, poor foam stability, lack in body due to a lower protein content in rice malt, especially nitrogen soluble compounds and FAN, which contribute to body and mouthfeel of beer [60]. Rice contains higher fat content than barley, leading to a greater susceptibility to oxidation of unsaturated free fatty acids and to the development of rancid notes [88]. Color is very pale, due to low kilning temperature (70 °C), but also to less nitrogen compounds available for Maillard reaction [59]. In a top-fermented rice beer the perceived attributes are caramel, cereal and vanilla. It results sour, probably due to the relatively low pH, and bit astringent [62]. There were used some strategies to improve rice beer quality. For example, the use of defatted rice bran (DRB) was able to impart a darker color, greater turbidity, greater foam volume and full-bodied structure perception [63]. Caramelized and dark rice malt were also used to increase flavor, color, body of rice beer and also its nutritional value, due to their high polyphenols content and a great antioxidant activity [132]. As reported by Diakabana et al. [65], final product obtained by corn malt has a low alcohol content and slightly bitter taste. The presence of unsaturated fatty acids in corn malt negatively affects the foam stability, but also due to low FAN and total nitrogen content. Corn also influences wort color and flavor. In fact, as demonstrated by Poreda et al. [90], color decreases by 1°EBC for each addition of 10% of corn grist. Sorghum beer has a higher color value, expressed in the EBC unit, compared with barley; indeed, sorghum is used mainly for “opaque beer” production. As reported by Yeo et Liu [130], sorghum beer is characterized by a slightly sweet taste, it is slightly sour due to the formation of lactic acid, with a mild touch of bitterness or astringency on the tongue. Foam collapses very fast [55]. Final product realized by malted or unmalted teff has an acceptable quality, in both cases. One hundred percent raw teff beverage has a fruity flavor, sweet taste and light body. On the other hand, teff malt gives the product notes of nuts, biscuits and vanilla. Foam stability is lower than barley beer, because of low total protein content. Color value is higher in product obtained by malted teff than raw, due to low production of Maillard reaction products during the boiling phase [74]. Brewing with tropical cereals like millet results in beer with different sensory characteristics than the conventional one. Millet malt has a different color and unusual flavor [56]. From a comparative study of experimental beers, brewed from millet, sorghum and barley malts, it is emerged that millet beer is darker than others, probably due to a high tannins content, better foam retention than sorghum beer, less alcohol content and a flavor that is not very reasonable [55]. Beer obtained by the use of buckwheat as adjunct have acceptable sensory characteristics. Beer color, bitterness and colloidal haze increase with increasing amount of buckwheat as adjunct, as well as for esters, responsible for fruity flavor. On the contrary, foam stability decreases, especially in 100% buckwheat beverage, due to low TSN and low high-molecular-weight proteins content [103]. Quinoa beverages have a different aroma, compared to conventional beer, because there is a lower production of the important volatile compounds, which determine the typical aroma of a conventional beer [133]. A slight bitter-taste is probably due to the presence of polyphenols, magnesium, sulphate, some amino acids or other bitter-tasting molecules. In a study Deželak et al. [76], sensory analysis was carried out. Quinoa beverages, compared to buckwheat, obtained a lower score for each sensory attribute, except for palatfulness. Purity of taste received the worst score, probably due to a high content of metal cations. When barley is substituted with quinoa seeds or flakes, alcohol content decreases, because quinoa produces more glucose than maltose. The concentration of 1-propanol, responsible for alcoholic flavor, decreases with increasing of quinoa concentration, just like isobutanol, linked to alcoholic/solvent flavor, and for 2-phenyletanol responsible for rose-like, flowery or honey-like flavor. Opposite trend occurs for 2- and 3-methylbutanols, which imparted solvent-like, malty and banana flavors. According to Kordialik-Bogacka et al. [80], beer with 30% of

quinoa flakes has good scores for all attributes (aroma, taste, mouthfeel, carbonation, bitterness and overall mark). Therefore, to obtain a product with reasonable characteristics, it is better using quinoa as an adjunct. Compared to barley malt beers, those produced with amaranth have reasonable attributes regarding smell, taste, bitterness quality and body, with some negative aspects [89]. In conclusion, one of the main disadvantages in brewing with GF cereals and pseudocereals is a different sensory quality of final product compared to traditional beer. The other previously described approaches have no relevant influence on sensory properties of final product. Enzymatic treatment promotes beer stabilization without influencing foam stability and beer flavor, as reported in Alkeroyd et al. [134]. Quality attributes depending on nitrogen content, especially foam stability and volatile compounds, are highly influenced by enzymatic treatment because of protein modification. The others sensory attributes, such as taste, aroma and mouthfeel, are not affected by this treatment. For example, AN-PEP treatment reduces gluten content below 20 ppm, maintaining an acceptable sensory properties [72,135]. With regard to beers obtained with the adjunct of enzymes extracted from germinated cereals, they have reasonable sensory properties, but unsuitable foam stability [135]. The opposite occurs for treated beers with mTG: their foam stability is not different from conventional beer; the same thing for beer flavor. There is a difference in turbidity of the sample due to the cross linking of proteins and presence of colloidal particles, and then also the color intensity increases with the increasing dose of mTG. The high turbidity of the sample may be resolved with filtration or sedimentation [120]. Precipitation treatment may influence aroma, taste, flavor, foam retention, bitterness and yeast fermentation performance [112]. As pointed out by Watson et al. [111], tannin acid, PVPP or silica gel application does not influence foam stability, unlike AN-PEP treatment. As reported in this review, there are different approaches to produce GF beer, but each one with its own technological and sensory issues. The high number of variables to be monitored suggest the importance of a tailored analytical approach in order to screen diverse experimental modalities, for example, proton transfer reaction combined with a time-of-flight mass spectrometer (PTR-ToF-MS) [136,137].

7. Microbiological Issues in GF Beer Production

Fermentation process reduces pH value of the wort due to yeast activity, with consequent development of acid compounds. pH reduction promotes the proteolytic activity and so also the proteolysis of gluten. However, some gluten peptides tend to remain in the final beer. Control of the fermentation progress is crucial for an efficient gluten reduction, and also the use of specific yeast strains may be a promising tool to enhance sensory attributes, especially flavor, of GF cereal products and beverages [138]. GF beer has higher production costs rather than conventional beer, because of a higher price market of alternatives grains and the following technological adaptations. Serial repitching of the yeast biomass can reduce production costs and the environmental waste produced at the end of fermentation. This approach was adopted by Deželak et al. [139]. They inoculated the *Saccharomyces pastorianus* strain to ferment GF buckwheat and quinoa wort. The extremely high metal levels of quinoa, especially iron and zinc, pseudocereals resulted toxic for the fermentation performance after two or three serial repitching. This did not appear for buckwheat [133]. Furthermore, the predominance of glucose rather than maltose affected the fermentation performance negatively. In fact, yeasts used in conventional fermentation usually prefer maltose rather than glucose. In this way, when pseudocereals are used instead of barley, by-products of fermentation will be different. As regards the impact of yeast strains' metabolism on aroma and flavor of the final product, Zarnkow et al. [99] evaluated the use of different yeasts, for example, *S. bayanus*, *S. cerevisiae* and *B. bruxellensis*, to improve aroma of millet beer and produce aroma compounds comparable to the conventional product. Aromatic profile obtained was very different because each yeast produced different compounds due to a different metabolism of fermentation. Another issue about brewing with raw GF materials was highlighted by Kruger et al. [140] during sorghum and maize beer production. Their bran contained a high level of phytate, a chelating agent, which formed insoluble complexes with metals, important for yeast nutrition. During germination phytases levels increased, phytate content of malted grains decreased,

but the improvement of yeast fermentation performance could not be specifically attributed to the effect of phytases [140]. These studies suggested that the use of GF cereals or pseudocereals in brewing process might affect yeast performance. The same problem occurs in craft brewing processes due to a possible uncontrolled development of spoilage microorganism [141]. Therefore, craft GF beers could have this double negative effect, as far as microbiological aspects. In addition, yeasts for GF beer must grow in gluten-free substrates, such as molasses, cane or beet sugar. Therefore, it is better to use dry yeasts unlike liquid form, because the latter may have been cultivated in no GF substrates, and so they could contaminate the beer [142].

8. Conclusions and Future Trends

Conventional beer is not allowed in the GF diet. Hence there is a need to produce GF beers, in order to meet the market demands. This review's purpose was to provide a summary of the different approaches for GF beer production, highlighting their technological and sensory issues. These strategies can be distinguished as follows: use of raw materials naturally free from gluten and gluten reduction treatments. According to a survey on 185 GF-producing breweries (both industrial and craft) from 24 different countries around the world, reviewed on the web, the first method mentioned above is more common compared to the second. In the first case, GF cereals and pseudocereals or no-grain materials are used to produce GF beers. These ones are considered safe for people with CD, because there is no gluten in the whole brewing process. The main GF raw materials used for this purpose are rice, maize, sorghum, millet, teff, quinoa, buckwheat and amaranth. Oat is also used in the brewing process, but there are still different opinions on its use in the GF diet. GF grains have some technological and sensory issues. The main question is their too high gelatinization temperature, not compatible with the optimal conditions for the enzymatic activity. Therefore, more studies are needed to optimize the malting conditions, in order to fit out the raw material for the brewing process. In addition, these GF grains give sensory properties different from those perceived in a conventional beer. Further work needs to be done on the development of the right formulation of a GF beer, taking into account both technological and sensory aspects. These GF grains should be used in combination among them, so as to offset the negative aspects related to the use of each raw material taken in isolation. This is one of the most important challenges. In the second case, conventional raw materials, such as barley or wheat, are used, and then gluten is removed by enzymatic or precipitation treatments. The final product produced is defined as GR beer. To date, AN-PEP are the most used enzymes in the brewing process, giving a beer with unchanged sensory characteristics, except for foam stability, which is an attribute influenced by total protein content. There are other ways to reduce gluten content below the safe threshold, too. For example, using other microbial enzymes or products as stabilizers in the brewing process. However, there are still doubts about the safe consumption of GR beers by celiacs, because gluten toxic peptides may remain in the final product, in a quantity exceeding the limit provided by regulation on GF products. The use of innovative analytical techniques for the determination of gluten in fermented foods, for example, LC-MS/MS, should be encouraged, in order to avoid false negatives. Further experimental investigations are needed to estimate the right dose of enzymes and stabilizers to use for gluten content reduction below 20 ppm, without excessively affecting the sensory quality of the final product, being this aspect a crucial point for its acceptability. Future perspective is the reduction of the immunogenic activity of gluten by genetic engineering techniques, in order to produce GF products containing barley or wheat without adding enzymes, additives or changing the process. An issue of the future will certainly be the optimization of the entire brewing process, providing breweries with well-established technologies in order to reduce the price of GF beers, which is still too high compared to conventional ones. Table 4 provides the principal strengths and weaknesses of each approach described in this paper.

Table 4. Approaches for gluten-free (GF) beer production: principal strengths and weaknesses.

Approaches	Strengths	Weaknesses
Use of GF cereals or pseudocereals	<ul style="list-style-type: none"> • Total absence of gluten in raw materials 	<ul style="list-style-type: none"> • High production costs due to high prize of raw material and technological adaptations • Low diastatic power • High gelatinization temperature • Need to add exogenous enzymes to improve brewing attitudes of GF grains • Different sensorial characteristics than traditional beer
No-grain materials	<ul style="list-style-type: none"> • Use of no-gluten containing ingredients • Possibility to add different kind of ingredients to enhance the quality of final product 	<ul style="list-style-type: none"> • Possible not acceptable sensory aspects
Enzymatic treatment	<ul style="list-style-type: none"> • Low dosages needed to reduce gluten content • Enzymes are considered Generally Recognized As Safe (GRAS) • Enzymes extraction is a well-established technique • Stables and actives at working T and pH • Easy to add during production process • Sensory quality of final beer is not heavily influenced 	<ul style="list-style-type: none"> • Foam stability is slightly influenced • Filtration or centrifugation at the end of the process increase production costs
Precipitation treatment	<ul style="list-style-type: none"> • Frequently used by brewers as stabilization treatment • Increased beer clarity 	<ul style="list-style-type: none"> • Less significant gluten reduction than enzymatic treatment • Impact on aroma of final beer
Genetic engineering and innovative approaches	<ul style="list-style-type: none"> • Ease in manipulate genes • Possibility to create a grain with low gluten content while maintaining unchanged brewing attitudes 	<ul style="list-style-type: none"> • Not legally accepted in all countries • Expensive techniques

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