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PREBIOTIC PROPERTIES OF BREWER'S SPENT GRAIN – LITERATURE REVIEW

S u m m a r y

Background. Brewer's spent grain (BSG) is a by-product of beer production, constituting approx. 85 % of all generated by-products in the brewing industry, primarily utilized for livestock feed. It is characterized by high levels of fiber (40 ÷ 60 %), protein (19 ÷ 30 %), and fat (10 %). The protein occurring in BSG contains all amino acids, including exogenous ones, which are not synthesized by the human body.

Results and conclusions. Brewer's spent grain fiber mainly comprises cellulose, hemicellulose and lignin. Due to various fiber fractions, including arabinoxylan (AXs), it is suspected to possess the ability to modulate the human gut microbiota. *In vitro* studies conducted so far have demonstrated that BSG and extracted AXs fractions stimulate the growth of health-promoting bacteria such as *Bifidobacterium* and *Lactobacillus*, however, they also have the capacity to stimulate bacteria of the *Enterobacteriaceae* family. Moreover, under the influence of brewer's spent grain, the abundance of *Bacteroides* and *Firmicutes* bacteria decreases, while the abundance of *Actinobacteria* increases. Additionally, it has been proven in each of the studies that the addition of BSG stimulates the synthesis of short-chain fatty acids, including propionic acid and acetic acid, with acetic acid being the most prominently affected. Brewer's spent grain may enhance the scavenging of free radicals due to the presence of phenolic compounds and increase the antioxidant activity of food. Further research, including studies utilizing a dynamic *in vitro* digestive system and *in vivo* investigations, is necessary to confirm the beneficial impact of BSG on human health.

Keywords: prebiotic properties, microbiota, fiber, arabinoxylan, short-chain fatty acids

Introduction

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For humans to sustain themselves, food is required to be produced, processed and consumed. Such operations involve the generation of by-products, and this frequently ends up in the waste stream. Also, every year, about 1.3 billion tons of food go to

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waste along the whole food production value chain due to final consumption, retail chain, industrial processing and agricultural operations [22]. Approximately 630 million tons of food go to waste in low-income countries mainly through losses of pre-and post-harvest, while over 670 million tons of food are discarded as waste from industrial processing and consumption [1].

Brewer's spent grain (BSG) stands as the primary brewing industry's by-product, accounting for approximately 85 % of the total generated by-products. The yearly BSG production in Europe, measured based on dry matter, was estimated in 2007 to be around 3.5 million metric tons. BSG boasts a rich composition, comprising lignin (also around 28 %), non-cellulosic polysaccharides (approximately 28 %) and cellulose (about 17%). Additionally, it contains significant quantities of polyphenols $(0.7 \div 2.0 \text{ g})$ / 100 g dry material) [26]. Because all bioactive compounds remain in the BSG during the brewing process, it is rich in minerals, vitamins, polyphenols, proteins and fiber. [15]. The BSG extracts support indirectly or directly the activities of anti-hypertensive, anti-microbial, anti-mutagenic and antioxidant capacity [5].

One of the ways to lessen biological waste is to utilize by-products like BSG as an additive to food. Various food processing techniques have been used to maximize BSG's nutritional potential. Moreover, the BSG food applications are in line with consumer desire for wholesome, plant-based food substitutes. With the right processing and bioprocessing techniques, the high nutrient content of BSG, which includes various forms of proteins, sugars, hemicelluloses and polysaccharides can be transformed into a variety of beneficial nutraceuticals and food for consumption by humans [22].

A biological nutrient group known as prebiotics undergo degradation by microorganisms within the gastrointestinal tract (GIT), notably by bacteria from the *Bifidobacterium* and *Lactobacillus* genus. When prebiotics are consumed, either as supplements or as an ingredient of food, the microbiota in the colon breaks them down and metabolizes to short-chain fatty acids (SCFAs). The SCFAs are not only released into the colon, but they also are absorbed into the bloodstream. Among the extensively studied prebiotics in terms of their impact on human health are galactooligosaccharides (GOS) and fructooligosaccharides (FOS). The qualification of a compound as a prebiotic is closely tied to its dietary fiber content [4]. However, other substances express prebiotic properties, for example, some polyphenols support the growth of beneficial bacteria [29].

Therefore, BSG holds promise as a natural carrier of prebiotics due to its rich composition. It contains polysaccharides and polyphenols that can fuel the growth of beneficial gut bacteria. Incorporating BSG into food products can introduce these prebiotic compounds, enhancing nutritional value and promoting gut health. This sustainable approach can reduce food waste, aligning with circular economy principles. However, successful integration requires addressing taste, texture and consumer acceptance through innovative food processing techniques. The use of BSG as a prebiotic carrier in food products not only supports gut health, but also advances sustainable food practices by repurposing a significant industry by-product [4].

The levels of polysaccharides and polyphenols suggest that BSG may have beneficial effects on gut health by promoting beneficial bacteria, supporting gut barrier integrity and potentially modulating microbial populations. However, extensive research is required to understand BSG's precise mechanisms on the gut microbiota and its overall impact on human health [11]. Therefore, the review aimed to search and analyze available data on the prebiotic properties of brewer's grain and the possibility of using it as as a functional food ingredient.

Methodology

Sections "Composition of the spent brewery grains" and "Utilization of spent brewery grains in food technology" of the review are based on the standard data search strategy. Scopus, Web of Science, PubMed and Google Scholar databases were used. For the section "Impact on the gut microbiota", the strategy with boolean operators was applied. The search began by finding primary research in electronic databases of scientific articles included in the protocols. The authors selected the studies using the inclusion and exclusion criteria based on titles, abstracts and full texts of articles. Only the papers that concerned the reaction of the microbiome to BSG supplementation or fiber fractions isolated from it were taken into account. Other studies in which gut microbiota were not the subject of a study were not included. Review works were also not included. The following words were used for the search: spent brewer* grain* AND microbiota AND prebiotic. The search was conducted on January 5, 2024. The word searching was adjusted to each database separately. Article titles and abstracts were reviewed for duplication across search engines. Finally, five articles were selected.

Results and discussion

Composition of the spent brewery grains

Fiber

The fiber fractions in BSG are hemicellulose, cellulose, lignin and a few other groups of chemicals, e.g. a fraction of glucans. The fiber content in BSG is $40 \div 60\%$ of dry matter. The content depends on the type of malt used and the technological parameters of the mashing process [14, 17].

Hemicellulose constitutes an essential part of spent grain fiber and can reach up to 40 % of dry matter [7]. It consists mainly of polysaccharide chains where sugar residues, mainly xylose, are linked by β-(1→4) bonds, with substitutes such as arabinate groups. These polysaccharides constitute arabinoxylans, which are the main fraction of hemicelluloses and are the largest selective fraction of dietary fiber in BSG. The detailed structure of AXs consists of a linear β -(1→4)-linked xylan skeleton to which α-larabinofuranose units are attached as side residues via α -(1→3) and/or α -(1→2) [29]. Arabinoxylans from BSG have a higher ratio of arabinose to xylose in their structure. This is due to more arabinose side chains than in the wheat AXs. Additionally, BSG contains mainly insoluble arabinoxylans originating from cell walls and bran. This is the mashing result, where most of the AXs soluble passes into the beer wort [25]. The content of cellulose in spent grain ranges from $12 \div 25$ % of dry matter. It includes long polysaccharide chains composed exclusively of glucose monomers connected by β -(1 \rightarrow 4) bonds and is an insoluble fraction of BSG fiber [17, 27]. While lignin constitutes approximately $10 \div 25$ % of the dry matter of BSG [2] and is classified as an insoluble non-carbohydrate fraction of dietary fiber [9]. Lignin is a complex, amorphous, three-dimensional, long-chain, aromatic polymer of high molecular weight, consisting of phenylpropanes, methoxy groups and non-carbohydrate polyphenolic substances linked by ether bonds [3, 23]. Moreover, BSG contains other fiber fractions, although their content is lower. These fractions include (1–3,1–4)-β-d-glucan. Monosaccharides, such as xylose, glucose, and arabinose, as well as trace amounts of rhamnose and galactose, are also present in the grain [14, 17]. The total fiber found in BSG is called lignocellulose and includes all the components discussed above except for monosaccharides [14].

Proteins

Proteins constitute approximately $19 \div 30$ % of the dry weight of BSG, and the most abundant are hordeins (prolamins), glutelins, globulins and albumins. These proteins function as storage proteins in barley seeds [14]. During the production of malt and mash, these proteins are degraded into smaller peptides and free amino acids by proteolytic enzymes [8, 9]. In terms of amino acid composition (Table 1), BSG protein is rich in glutamine/glutamic acid, valine and leucine, while the amino acid content of cysteine and methionine is low [9, 28]. Amino acid composition in BSG depends on the type of malt used and technological and storage conditions [14].

Lipids

Lipids constitute approximately 10 % of the dry matter of BSG [19]. The dominant lipids are triglycerides, which include about 65 % of total lipids, and free fatty acids, which account for about 20 % of total lipids [10, 21]. The main fatty acids in BSG are linoleic, palmitic and oleic acids. BSG also contains monoglycerides and diglycerides (10 % of total lipids) [21]. Importantly, BSG contains steroid compounds, including phytosterols, which are present in significant amounts in BSG (approx. 5 % of total lipids). The most abundant sterols are free and conjugated variants [10, 21].

The high content of phytosterols is important for health and nutritional reasons in this by-product [21].

Amino acids Aminokowasy	g/100 g	
	Waters et al. 2012 [28]	Connolly et al. 2013 [9]
Alanine / Alanina	4.1	4.3
Arginine / Arginina	4.5	6.0
Asparagine / Asparagina	1.5	6.6
Aspartic acid / Kwas asparaginowy	4.8	
Cysteine / Cysteina		1.4
Glutamic acid/ glutamine Kwas glutaminowy/glutamina	16.6	24.7
Glycine / Glicyna	1.7	3.8
Histidine / Histydyna	26.2	3.6
Isoleucine* / Izoleucyna	3.3	4.2
Leucine* / Leucyna	6.1	7.2
Lysine* / Lizyna	14.3	3.2
Methionine* / Metionina		1.4
Phenylalanine* / Fenyloalanina	4.6	6.2
Serine / Seryna	3.8	9.7
Threonine* / Treonina	0.7	4.1
Tryptophan* / Tryptofan	0.1	\blacksquare
Threonine / Treonina		3.2
Tyrosine / Tyrozyna	2.6	3.5
Valine* / Valina	4.6	6.0

Table 1. Amino acids composition of spent brewer's grains protein Tabela 1. Zawartość aminokwasów w białku młóta browarnianego

Utilization of spent brewery grains in food technology

Until now, BSG has not been extensively utilized in food technology. However, several studies were conducted to analyze the impact of adding BSG on the organoleptic and sensory characteristics of bakery and flour-based products. These studies encompassed the production of bread, breadsticks, dough and pasta.

In one particular study, authors investigated the effect of adding 5 % and 10 % BSG on the sensory properties of bread, breadsticks and pizza dough. Control samples consisted of traditional recipes with a low amount of fiber. The study observed a decreased specific volume of products prepared with the addition of BSG, attributed to reduced extensibility and weakening of the gluten connections. The addition of BSG led to a significant change in color intensity, where the brightness parameter (L^*) decreased substantially with an increasing amount of brewer's spent grain. BSG addition also affected the parameters (a^*) and (b^*) . The addition of BSG hurt the sensory characteristics of the samples, limiting their acceptability [2].

Ktenioudaki et al. [16] realized the study focused on the effect of 15 %, 25 % and 35 % addition of BSG on the nutritional properties of bread sticks.. It was demonstrated that the addition of BSG significantly increased the protein content in the product, which was 14.3 % for the control sample and 18.4 % for the sample with 35 % BSG. The fiber content also significantly increased, i.e. 6 % for the control sample and 27 % for the sample with 35 % BSG. Similarly to the previously mentioned study, all color parameters underwent changes. The volume of the baked goods decreased, and their hardness was reduced.

Neylon et al. [20] analyzed the impact of BSG on the quality of pasta, a weakening of gluten properties was again demonstrated compared to the traditional control sample. However, these properties were more favorable than those observed in wholegrain pasta samples, which are currently becoming increasingly popular. It was demonstrated that pasta with the addition of BSG exhibited higher fiber content, lower glycaemic index, increased strength and firmness compared to the control sample.

Impact on the gut microbiota

Five articles examining the impact of BSG on the human gut microbiota through the utilization of donor faecal samples were identified. All studies were conducted *in vitro*, employing BSG and arabinoxylan extracts sourced from BSG. The characteristics of its main results are presented in Table 2.

In the study carried out by Bonifácio-Lopes et al. [5], BSG subjected to extraction using Solid-Liquid Extraction (SLE) and Organic Hydro-Solvent Extraction (OHE) methods were employed, followed by enzymatic digestion utilizing enzymes representative of those present in the human gastrointestinal tract. Microbiome content characteristics were obtained using Reverse Transcription Polymerase Chain Reaction (RT-PCR method). The obtained outcomes were compared with the growth of the analyzing group of bacteria on a negative control representing the MRS microbial medium and a positive control consisting of MRS medium supplemented with fructooligosaccharides (FOS). The study demonstrated that extracts from BSG stimulated the growth of bacteria belonging to the genera *Bifidobacterium*, *Lactobacillus* and *Enterococcus*, with growth comparable to the positive control containing FOS. Regarding *Bacteroides spp.*, a $22 \div 36$ % increase in bacterial abundance was observed within the first 12

- Table 2. Characteristics of studies included in the analysis of the impact of spent brewery grains on microbiotacomposition and SCFA levels change
- Tabela 2. Charakterystyka badań włączonych do analizy wpływu młóta browarnianego na zmiany składu mikrobioty i stężenia SCFA

hours, followed by a significant reduction at 24 and 48 hours compared to the negative control. Similar results were observed for *Clostridium leptum*, which exhibited an initial increase in abundance after 12 hours of incubation, followed by a subsequent decrease. Incubation with BSG extract led to an overall decrease in the abundance of bacteria from the *Bacteroidetes* and *Firmicutes* phyla. The *Firmicutes*/*Bacteroidetes* ratio increased by $15 \div 22$ % during the initial 12 hours of incubation, followed by a subsequent decline, but still maintaining a ratio >1.0 . During the incubation, an increase in the synthesis of short-chain fatty acids (SCFAs) was observed, although the growth was lower than in the control sample with FOS. The most pronounced stimulation of synthesis was demonstrated for propionic, acetic and succinic acids. An increase in the synthesis of butyric acid was also observed, although this result did not reach statistical significance. The study also revealed an elevation in the total content of phenolic compounds, resulting in an enhanced antioxidant activity during *in vitro* digestion simulation [5].

In the study conducted by Lynch et al [18], experimental material was prepared from BSG by extracting arabinoxylans (AXs) through a process involving saccharification and fermentation with *Lactiplantibacillus plantarum* F10. Six different extract variants were prepared by employing various extraction parameters. Additionally, untreated BSG without modification and commercial AXs were analyzed as control samples. Before scrutinising the microbiome changes, the samples underwent *in vitro* digestion simulating enzymatic conditions present in the stomach and small intestine. Microbiome changes were analyzed using faecal samples from six healthy donors. After fermentation, genetic material was extracted from the samples and subsequently subjected to 16S sequencing. Fermentation of BSG without modification resulted in a significant tenfold decrease in the abundance of *Bacteroidetes* bacteria and a twofold increase in *Lactobacillus* and *Actinobacteria* bacteria. A similar decrease in the abundance of *Bacteroidetes* bacteria was observed in all extract samples, but not in the commercial AXs. Most extract samples stimulated the growth of *Lactobacillus* bacteria. BSG slightly stimulated the growth of *Bifidobacterium* bacteria, although this stimulation was greater in the case of extract analyses. All samples also stimulated the growth of *Bacteroides* bacteria, as well as *Escherichia - Shigella* bacteria belonging to the *Proteobacteria* phylum. The majority of analyzed samples, including BSG and commercial AXs, stimulated the synthesis of propionic acid, acetic acid and total SCFAs. An increased synthesis of butyric acid was demonstrated only for commercial AXs. For all analyzed samples, a decreased synthesis of isovaleric acid, isobutyric acid and total branched-chain fatty acids (BCFA) was observed. Only BSG stimulated the synthesis of valeric acid [18].

Calvete-Torre et al. [6] used BSG samples without modification and extracts of AXs derived from them. The samples underwent *in vitro* digestion before faecal fermentation. Faecal samples from three healthy donors and three donors with Crohn's disease (CD) were used in the experiments. Microbiome changes were analyzed using the 16S sequencing method. The authors demonstrated that the majority of analyzed samples significantly stimulated the growth of bacteria from the *Escherichia-Shigella* genera in microbiota derived from healthy donors, while bacteria from the *Clostridium* genus were strongly stimulated in the microbiota of CD's patients. Bacteria from the *Eubacterium*, *Bifidobacterium, Parasutterella* and *Dorea* genera increased in abundance in the microbiota of healthy individuals only after fermentation with one of the extract samples (AS-AX 4). After the fermentation of faecal samples from healthy individuals with the addition of the analyzed samples, bacteria from the *Caprococcus*, *Lachnoclostridium* and *Bilophila* genera also experienced an increased growth. The fermentation of faecal samples from patients with CD with the addition of the analyzed samples resulted in an increased abundance of bacteria from the *Senegalimassilia*, *Collinsella*, *Phascolarctobacterium* and *Parabacteroides* genera. Regarding the impact of BSG samples on the modulation of gut microbiota, a negative correlation was observed in the study between the abundance of *Bifidobacterium* and *Escherichia-Shigella* bacteria in samples from both healthy and CD's patients. The fermentation of faecal samples with BSG from healthy donors showed a positive correlation between *Parasutterella*, *Bifidobacterium* and *Dorea*, and a negative correlation between *Bilophila* and *Eubacterium halli*. In the case of BSG samples fermented with faecal samples from patients with CD, weaker associations were observed between health-promoting bacteria, which may be related to existing dysbiosis. In the microbiota isolated from healthy individuals, there was an increase in the commensal bacterial groups present in the eubiotic microbiota type, while no such change was observed in the dysbiotic microbiota obtained from CD's patients. It is suspected that additional supplementation in these individuals with probiotic strains could complement a potential synbiotic therapy to restore intestinal eubiosis in these individuals. The synthesis of all analyzed SCFAs was significantly higher after fermentation with samples from healthy donors compared to samples from patients with CD. However, no significant differences were observed in the stimulation of SCFAs synthesis between BSG and its extracts [6].

Gómez et al. [13] used BSG subjected to the extraction process of AXs in their study. The study did not analyze unaltered BSG. To analyze changes in the microbiota, faecal samples were obtained from three elderly healthy donors (age > 60 years old). Changes in the bacterial population of faecal samples were analyzed using the Fluorescence In Situ Hybridization (FISH) method and probes targeting 16S rRNA. The control sample consisted of a medium without an added carbon source. The fermentation of samples containing AXs extracted from BSG resulted in a significant increase in bacteria from the *Lactobacillus*/*Entrococcus, Bifidobacterium* and *Bacteroides*/ *Prevotella* genera. The total bacterial count also increased. The abundance of *Clostridium histolyticum* bacteria decreased during incubation compared to the control sample. These changes occurred after seven hours of fermentation, intensified with prolonged incubation, and were similar to the effects observed for FOS. Regarding the results concerning changes in SCFAs levels during 45-hour incubation, compared to the control sample, the content of acetic acid and the total SCFAs increased almost threefold, the content of propionic acid increased over twofold, and the content of butyric acid increased over threefold [13].

Reis et al. [24] employed two AXs extracts from BSG, prepared using ultrasonic extraction (AX1) and alkaline extraction (AX2), for their research. Faecal samples were obtained from three healthy donors, and the abundance of the analyzed bacteria was assessed using an RT-PCR method. In the analysis of changes occurring in the faecal samples from the first donor with the addition of the AX1 sample, there was an increase in total bacteria and *Bacteroides*/*Prevotella*. However, there was no increase in the abundance of *Bifidobacterium*. On the other hand, the application of the AX2 sample led to an increase in *Bifidobacterium*, *Bacteroides*/*Prevotella* and total bacterial count. This increase was higher than in the control samples and those with the addition of FOS. In the faecal samples from the second donor, after the application of both extract variants, there was an increase in the abundance of *Bacteroides*, *Bifidobacterium*, *Clostridium*/*Eubacterium* and total bacterial count. However, in the case of the AX2 sample, the increase was stronger than in the case of the AX1 sample. The faecal samples from the third individual showed the highest increase in all analyzed bacteria, especially *Bifidobacterium*, with the AX2 sample resulting in the most substantial increase in bacterial abundance. Both variants of the samples significantly increased the synthesis of SCFA, but the AX1 sample exhibited the strongest effect [24].

Conclusions

Brewer's spent grain is a post-production raw material with high nutritional value. High protein and fiber content can exhibit an important modulatory effect on the intestinal microbiota. The fiber present in BSG is composed of multiple fractions that can modulate the growth of a wide range of microorganisms belonging to different families and genera. A valuable fiber fraction appears to be arabinoxylan, which can enhance the prebiotic effect of the analysed material.

Studies indicate that brewer's spent grain can influence human gut microbiota. Consistent findings reveal that BSG promotes the growth of beneficial *Lactobacillus* and *Bifidobacterium* species bacteria, potentially demonstrating health-promoting properties. However, brewer's spent grain also stimulates the growth of many other groups of bacteria including *Dorea, Coprococcus, Bacteroides, Prevotella* species, and particularly the *Enterobacteriaceae* family. All these groups of bacteria are present in adequate amounts in the intestinal microbiota of healthy individuals. An unbeneficial observation from the study may be a significant increase in the *Enterobacteriaceae* bacteria family. It may be due to methodological issues and flaws in the *in vitro* methods or the composition of the SBG present in the study by Calvete-Torre et al [6]. However, BSG is identified as a potential prebiotic by-product. Although the current research focuses on faecal fermentation under static *in vitro* conditions, further investigations using dynamic digestive systems and *in vivo* studies are essential to confirm BSG's impact on human microbiota and health.

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WŁAŚCIWOŚCI PREBIOTYCZNE MŁÓTA BROWARNIANEGO – PRZEGLĄD LITERATURY

Streszczenie

Wprowadzenie. Młóto browarniane jest produktem ubocznym z produkcji piwa stanowiącym około 85 % wszystkich generowanych odpadów poprodukcyjnych w przemyśle browarniczym, dotychczas wykorzystywanym do karmienia bydła. Cechuje się dużą zawartością błonnika (40 ÷ 60 %), białka (19 ÷ 30 %) i tłuszczu (10 %). Białko obecne w młócie browarnianym zawiera wszystkie aminokwasy, w tym egzogenne, które nie są syntetyzowane przez organizm człowieka.

Wyniki i wnioski. Błonnik młóta browarnianego składa się głównie z celulozy, hemicelulozy oraz ligniny. Ze względu na poszczególne frakcje błonnika, w tym arabinoksylany podejrzewa się, że wykazuje zdolność do modulacji mikrobioty jelitowej człowieka. W dotychczas przeprowadzonych badaniach *in vitro* dowiedziono, że młóto browarniane i frakcje wyekstrahowanego arabinoksylanu stymulują wzrost bakterii potencjalnie prozdrowotnych z rodzaju *Lactobacillus* i *Bifidobacterium*, jednakże ma również zdolność do stymulacji bakterii z rodziny *Enterobacteriaceae*. Dowiedziono, że pod wpływem młóta browarnianego zmniejsza się liczebność bakterii typu *Firmicutes* oraz *Bacteroidetes*, zaś zwiększa liczebność bakterii z typu *Actinobacteria*. Ponadto, w każdym z badań dowiedziono, że dodatek młóta browarnianego stymuluje syntezę krótkołańcuchowych kwasów tłuszczowych, w tym najsilniej kwasu octowego i kwasu propionowego. Młóto browarniane może zwiększać wychwyt wolnych rodników przez obecność związków fenolowych i zwiększenie aktywności antyoksydacyjnej żywności. W celu potwierdzenia korzystnego wpływu młóta browarnianego na zdrowie człowieka konieczne są dalsze badania, w tym z wykorzystaniem dynamicznego układu trawiennego *in vitro* oraz badania *in vivo*.

Słowa kluczowe: właściwości prebiotyczne, mikrobiota, błonnik, arabinoksylany, krótkołańcuchowe kwasy tłuszczowe