



## Pigmented cereals and legume grains as healthier alternatives for brewing beers

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### ARTICLE INFO

#### Keywords:

Functional beverages  
 Craft beers  
 Pigmented beers  
 Legume beers  
 Antioxidants

### ABSTRACT

Pioneering studies were carried out on red-to-black pigmented grains for brewing beers. Together with pigmented cereals, the use of pulses was also proposed for making low-alcohol, gluten-free beers with improved contents of health-promoting compounds. So far, a new concept of beer is emerging among scientists, brewers, and consumers by expanding the assortment of conventional beer's ingredients. Coloured (red, purple, blue, black) cereal grains and legume grains are an untapped resource of functional compounds, each denoting different and complementary beneficial effects on human health. Among others, polyphenols contribute to protect against non-communicable diseases such as hypertension, heart diseases, cancers, diabetes and obesity. In this review, we summarized the improvement in terms of phenolic compounds and antioxidant capacity of beers obtained by using pigmented cereal grains and pulses as raw ingredients versus traditional beers. We examined the influence of these alternative materials on the qualitative properties of the beers. Moreover, we reviewed the contribution of traditional and non-conventional yeasts on the flavour and quality of these new functional beers. Finally, we discussed the use of different and complementary chemical methods for monitoring the composition, organoleptic profile, safety, and authentication issues with the aim to highlight the most promising to protect and promote novel beer products.

### 1. Introduction

Beer is one of the oldest alcoholic drinks around the world, dating back to Neolithic times and the Mesopotamians around 6000–7000 years ago (Vriesekoop, 2017). Nowadays, beer is among the most popular alcoholic beverage worldwide, with 1789.019 million hl produced mainly in China, USA, Brazil, and Mexico. A great part of beer's popularity and success lies upon its good taste and flavour and the lower costs compared to other traditional drinks (Pereira de Moura & Rocha dos Santos Mathias, 2018; Sohrabvandi et al., 2010). In the recent years, consumers are rediscovering this beverage also as a source of functional compounds including vitamins, minerals, and antioxidants (Salañã et al., 2020).

The main steps of the brewing process include malting, milling, mashing, boiling, cooling, fermentation, maturation, filtration, and

carbonation followed by microbiological stabilization, and packaging. Indeed, several changes were introduced in the latest years giving rise to diverse types of beer and beer-like beverages (Veljović et al., 2015). While in traditional beer-producing countries like Germany, only four ingredients are used for brewing, including water, malted barley, hops (*Humulus lupulus* L.) and yeast (Salañã et al., 2020), in other countries, the laws that regulate beer production are less stringent, allowing brewers to introduce some variations. In place of or together with barley, other carbohydrate sources (malted or unmalted; Humia et al., 2019) are used, such as wheat, rice, rye, oats, maize, sorghum, millet, and cassava, whose pros and cons in their use are indicated in Table 1.

The various modifications that were introduced over the time contributed to different sensory and nutritional properties of the beers (Cadenas et al., 2021). Especially craft beer producers are steadily opening the road towards the diversification of beer's offer for what

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<https://doi.org/10.1016/j.fbio.2023.102463>

Received 17 January 2023; Received in revised form 1 February 2023; Accepted 3 February 2023

Available online 4 February 2023

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**Table 1**

Technological pros and cons of the addition of pigmented adjuncts for beer production.

Pigmented Adjuncts	Pro	Cons
Corn	Grainy flavor sweetness	Low protein;
Oats	Silky texture	Overuse can bring astringency
Rice	Dry, clean taste	Low protein
Rye	Fruity, spicy, oily flavors	High glucans; whole rye very water absorbent,
Wheat	Malty spiciness	High in protein, can throw chill haze;
Legumes	Reduction in gluten and alcohol content	Reduced fruity flavor

concerns both the taste and the health properties (Habschied et al., 2020).

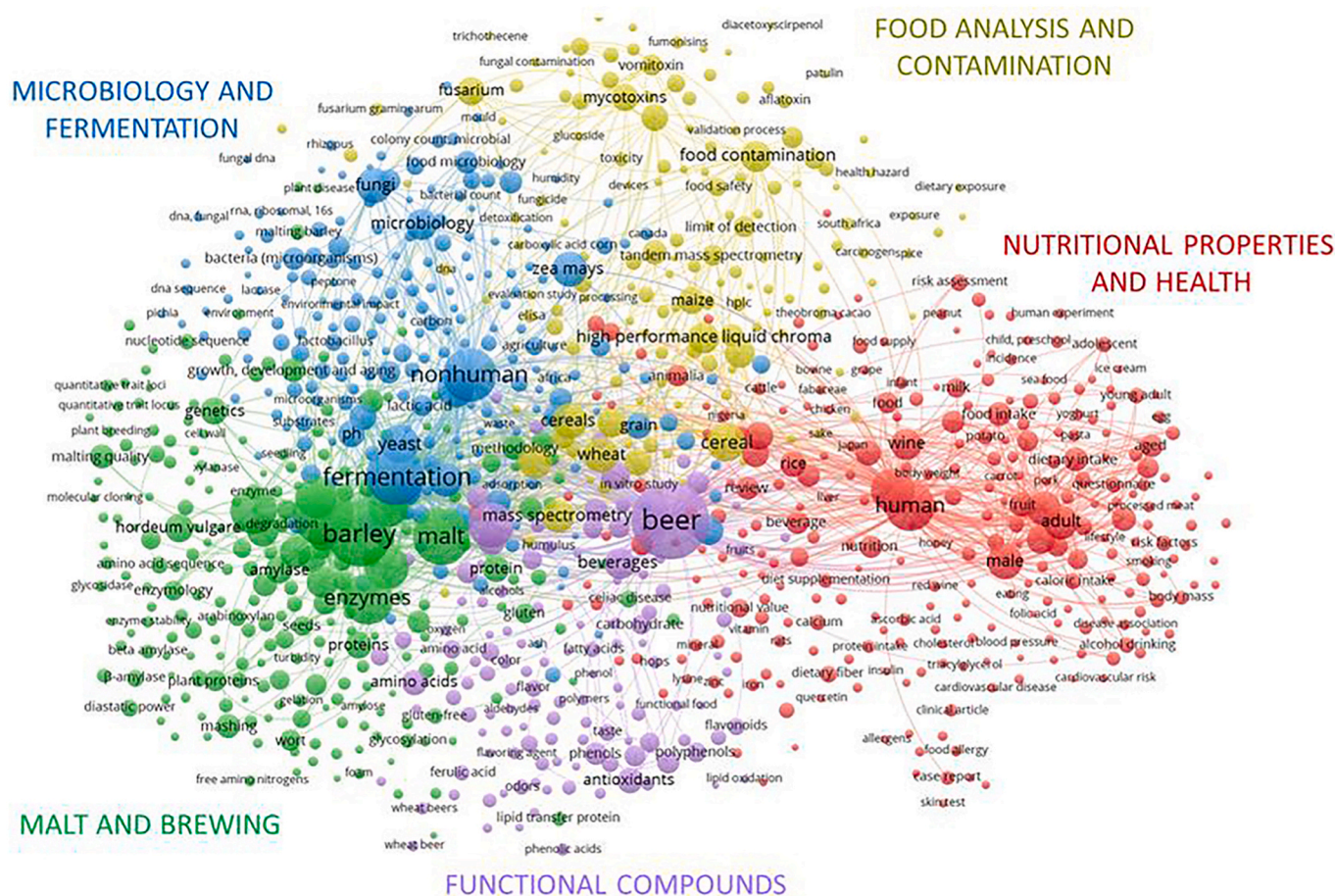
Pioneering studies focused on using pigmented cereals and pulses as alternative raw materials (Black et al., 2019; Luneia et al., 2018; Romero-Medina et al., 2020). Pigmented cereals are rich source of polyphenols that, besides having beneficial effects on human health, may contribute to beer quality influencing the colour, clarity, astringency, bitterness, flavour, and aroma (Panche et al., 2016). However, the potential health benefits associated with the consumption of functional beer are limited by the negative effects of alcohol and calories content (De Gaetano et al., 2016; Mellor et al., 2020). It is important to emphasise the need for moderate beer consumption as excessive intake of this beverage can lead to the development of chronic diseases and

other serious human health problems (World Health Organization, 2022). Therefore, several literature studies afforded the issue of enhancing the bioactive compounds of beer but also reducing the alcohol and calories content using novel ingredients such as pulses (Black et al., 2019).

After a bibliometric mapping of malt and beer production and reviewing the recent use of pigmented cereals and pulses as raw ingredients, this review reports the ability of coloured cereals and legume grains in improving the health-promoting, nutritional and sensory properties of beers also based on the microbial species used in fermentation. Some final remarks are given about the importance of quality controls and authentication of new beer types, especially craft beers.

## 2. Bibliometric mapping of malt and beer production research

Based on bibliometric mapping (Ellegaard & Wallin, 2015), we retrieved a total of 5838 published papers from the Scopus database for the period 1981–2022. The search was carried out based on selected keywords (i.e. malt, beer, and the crops names used for brewing). Considering the period comprised between the years 1981 and 2000, the number of documents per year varied from 28 to 99, with a mean of 57.6 papers per year. The average number per year increased to 113.2 in the period comprised between 2001 and 2005, being even higher (286.2) in the subsequent period (2016–2022). We identified five leading sources among the Journals (i.e. Journal of the Institute of Brewing, Journal of the American Society of Brewing Chemists, Journal of Agricultural and Food Chemistry, Food Chemistry, Journal of Cereal Science), each publishing more than one hundred papers. We also found that one



**Fig. 1.** Terms map based on malt and beer publications from 1981 to 2022. Different colors represent the terms (keywords) belonging to different clusters. The size of the nodes (circles) is related to the number of occurrences, while the links between nodes indicate the co-occurrence between keywords. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hundred and twenty-six countries, identified by authors' affiliations, published documents during the period 1981–2022, and sixty-two countries participated in at least ten papers, thus indicating the worldwide interest in malt and beer research. The five countries contributing with the highest number of documents were: United States ( $n = 811$ ; 11.6%), United Kingdom ( $n = 473$ ; 6.7%), Germany ( $n = 441$ ; 6.3%), China ( $n = 423$ ; 6.0%), and India ( $n = 320$ ; 4.6%).

The cluster analysis of terms included in the keywords, title, and abstract of the 5838 documents, was carried out by the software VOSviewer (Van Eck & Waltman, 2010) and the results are shown in Fig. 1. A total of 1000 keywords, with a minimum number of twelve occurrences, grouped into five clusters giving an overview of the structure of five hot research themes, each represented in green, blue, yellow, red, and violet colors (Fig. 1). The green cluster (“malt” and “brewing”) includes papers focusing on different topics, such as the replacement of barley malt with alternative carbohydrate sources; the addition of enzymes for wort production; the use of beer adjuncts to meet consumers tastes and impact on beer characteristics; genome mapping of grain and malt quality components; valorization of brewing by-products (Bogdan & Kordialik-Bogacka, 2017; Gupta et al., 2010). The blue cluster (“microbiology” and “fermentation”) includes literature studies investigating on the biology of brewing yeast; microbial ecology and cereal fermentation management; strain characteristics of *Saccharomyces cerevisiae* in the production of fermented food and beverages; growth of probiotic *Lactobacilli* strains in cereal-based substrates; release of compounds during fermentation and brewing (Bokulich & Bamforth, 2013). The yellow group (“food analysis” and “contamination”) refers to the qualitative-quantitative analysis of malt, beer, and other foods; beer and hop chemistry; food contamination by mycotoxins, heavy metals and synthetic particles; toxicology and risk assessment of mycotoxins in food and beverages; the impact of food processing and detoxification

treatments on mycotoxin contamination (Pinotti et al., 2016). The red cluster (“nutritional properties” and “health”) groups the studies that were carried out to establish the relationship between beer's alcohol and non-communicable diseases; beers and celiac disease and sensitivity; fermented foods as a dietary source of live organisms; biogenic amines, food safety and human health; beer constituents as potential cancer chemo-preventive agents; manufacturing process and beer acceptability (de Valois Lanzarin et al., 2021). Finally, the violet cluster (“functional food”) includes the papers investigating the antioxidant composition and activity of cereals and malt extracts; the role and significance of specific phenolic compounds in functional foods and beverages; the dietary intake and bioavailability of polyphenols from beers; the bio-functional components in processed pre-germinated cereals grain; prebiotic effects of cereal polymers (i.e. arabinoxylans) cross-linked with polyphenols present in beers (Habschied et al., 2020).

Fig. 2 shows the keywords occurrence per year and the number of publications average per year. In particular, the blue and dark green colour circles reveal that, early publications were characterized by a higher frequency of keywords focusing on: the use of tropical cereals for lager beer brewing; fermentation; bacteriocines; malting temperature and mashing methods influencing wort composition and beer flavour; ochratoxin contamination of barley and malt quality;  $\alpha$ -amylase and malt  $\beta$ -glucanase activity; long-term storage effects on beer quality (Gupta et al., 2010). Conversely, the most recent publications were characterized by some new keywords including “sensor analysis”, “food grain”, “phenols”, “flavor compounds”, “volatile organic compounds”, “antioxidant capacity”, “craft beer”, “obesity”, and “gluten free” that are represented in yellow and light green in Fig. 2. Many of these papers aimed at diversifying beer's flavour through the addition of medicinal or aromatic herbs and fruits; producing low-alcohol beers and alcohol-free beers; developing gluten-free beers and healthier beers with low

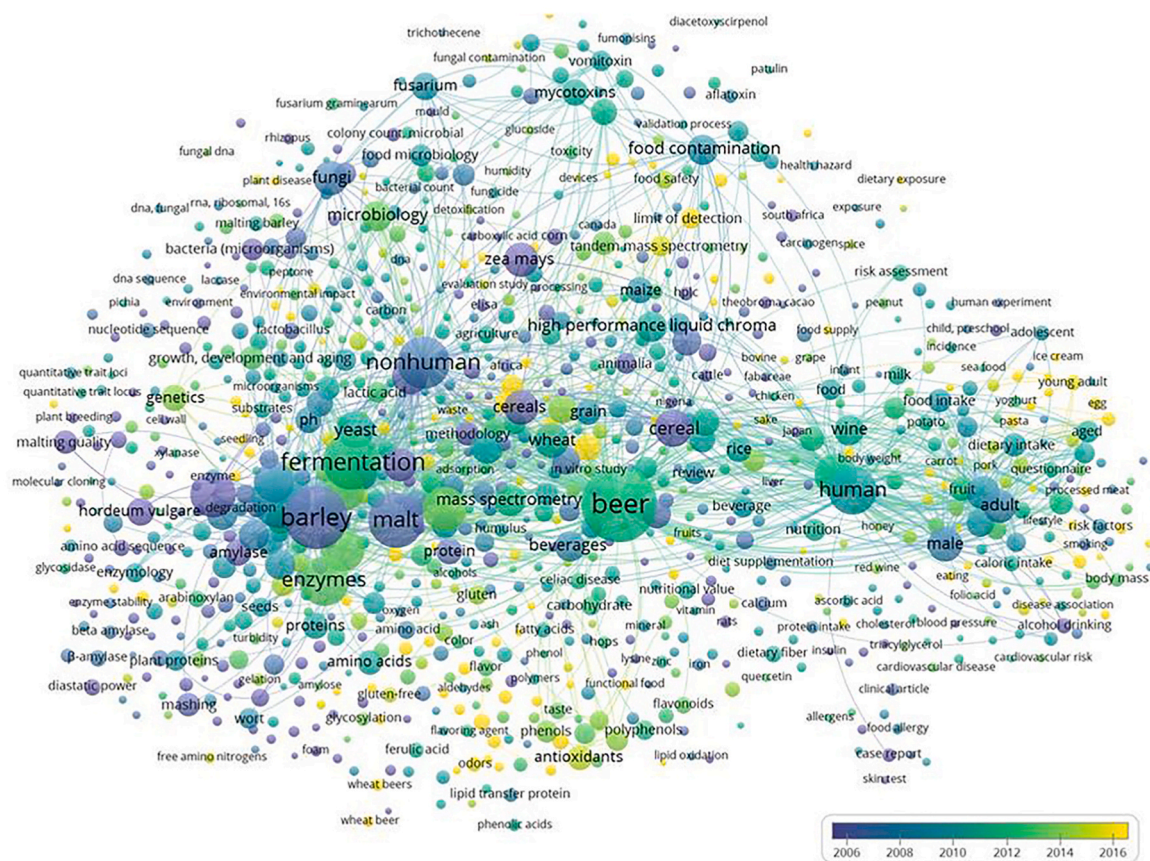


Fig. 2. Keywords year map based on malt and beer production publications. The blue, green, and yellow colors represent, respectively, terms mostly present in early, medium, and recent scientific documents. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

carbohydrate content, and higher contents of polyphenols and beta-glucans (Habschied et al., 2020).

### 3. Actual use of pigmented cereal grains for brewing beers

The use of pigmented cereals in beer brewing is quite recent and appears as an opportunity to diversify the beer's market and improve the profile of nutritional and nutraceutical components (Bassolino et al., 2022; Radonjić et al., 2020).

The colour of cereal grains is due to natural pigments such as carotenoids, anthocyanins and other phytochemicals that, due to their health-promoting significance, have become popular among consumers (Bassolino et al., 2022; Dwivedi et al., 2022). Yellow-amber colour is the most common in cereal grains which is due to the accumulation of carotenoids in the endosperm cells, whereas red colour depends on proanthocyanidins and phlobaphene occurring in the pericarp (Lachman et al., 2017; Rauf et al., 2019). Less cultivated are anthocyanin-rich cereals with purple, blue and black grains (Garg et al., 2016; Padhy

et al., 2022).

Pigmented cereal grains contain significantly large quantity of total anthocyanin compounds (TACs) ranging from 68 to 4700 µg/g in coloured rice, 10–305 µg/g in purple wheat and 17–211 µg/g in blue wheat, 35–84 µg/g in blue barley and 573–679 µg/g in purple barley, 27–1439 µg/g in coloured maize (see review Francavilla & Joye, 2020). Several investigations have found consistent correlation between TAC, total phenolic content, and the overall antioxidant potential (Harakotr et al., 2014; Lee et al., 2013; Lopez-Martinez et al., 2009). A higher antioxidant activity of TAC than vitamins C and E was even observed in an *in vitro* study in coloured barley germplasm (Kim et al., 2007). Overall, in comparing the effects of pigmented grains compared to white grains it is necessary to consider that grain yield and quality of cereal crops are typical quantitative traits influenced by several environmental factors, genotype × environment interactions, and the effect of the different technological processes to produce end-products (Gaikwad et al., 2020; Kaya & Akcura, 2014; Laddomada et al., 2021; Romano et al., 2022; Shah & Wu, 2019). Indeed, when several environments were considered

**Table 2**

Anthocyanin profile of red, purple, blue, and black of corn, wheat, rice, and barley grains. The contents of each individual anthocyanin are expressed as µg/g dry matter.

Grain color	Corn	Wheat	Rice	Barley	References
<b>Blue</b>	Cyanidin-3-glucoside (110.2 µg/g)	Malvidin-3-glucoside (5.5 µg/g)		Cyanidin-3-glucoside (10.7 µg/g)	Abdel-Aal et al., 2006; Sharma et al., 2020; Jin et al., 2022
	Cyanidin succinyl glucoside (30.9 µg/g)	Delphinidin-3-galattoside (4.9 µg/g)		Petunidin-3-glucoside (6.0 µg/g)	
	Cyanidin malonyl glucoside (13.2 µg/g)	Cyanidin-3-glucoside (4.5 µg/g)		Delphinidin-3-glucoside (3.9 µg/g)	
	Pelargonidin-3-glucoside (12.1 µg/g)	Petunidin-3-glucoside (3.2 µg/g)		Delphinidin (3.3 µg/g)	
		Delphinidin-3-rutinoside (2.1 µg/g)		Pelargonidin-3-glucoside (0.6 µg/g)	
		Cyanidin-3-rutinoside (0.6 µg/g)		Malvidin-3-glucoside (0.6 µg/g)	
		Delphinidin-3-glucoside (0.4 µg/g)			
<b>Red</b>	Cyanidin-3-glucoside (284.5 µg/g)	Cyanidin-3-glucoside (4.0 µg/g)	Cyanidin-3-glucoside (14.0 µg/g)	Cyanidin-3-glucoside (4.0 µg/g)	Abdel-Aal et al., 2006; Ficco et al., 2014; Ryu et al., 2003
	Peonidin-3-glucoside (58.7 µg/g)		Peonidin-3-glucoside (2.5 µg/g)		
	Cyanidin succinyl glucoside (43.5 µg/g)		Cyanidin diglucoside (1.7 µg/g)		
	Pelargonidin-3-glucoside (27.7 µg/g)		Cyanidin-3-rutinoside (1.3 µg/g)		
<b>Purple</b>	Cyanidin-3-glucoside (298.9 µg/g)	Cyanidin-3-glucoside (2.6 µg/g)	Cyanidin-3-glucoside (4172 µg/g)	Cyanidin-3-glucoside (96.9 µg/g)	Abdel-Aal et al., 2006; Sharma et al., 2020; Jin et al., 2022
	Cyanidin succinyl glucoside (101.6 µg/g)	Pelargonidin-3-glucoside (1.9 µg/g)	Peonidin-3-glucoside (59 µg/g)	Delphinidin (3.3 µg/g)	
	Cyanidin disuccinyl glucoside (57.6 µg/g)	Malvidin-3-glucoside (1.3 µg/g)		Pelargonidin-3-glucoside (2.0 µg/g)	
	Pelargonidin-3-glucoside (55.3 µg/g)	Delphinidin-3-galattoside (0.4 µg/g)		Cyanidin (1.8 µg/g)	
	Cyanidin malonylsuccinyl glucoside (35.1 µg/g)	Cyanidin-3-rutinoside (0.2 µg/g)		Petunidin-3-glucoside (0.5 µg/g)	
	Cyanidin malonyl glucoside (34.8 µg/g)				
Luteolinidin glucoside (31.0 µg/g)					
<b>Black</b>	Pelargonidin (969.7 µg/g)	Delphinidin-3-galattoside (29.1 µg/g)	Cyanidin-3-glucoside (2013.0 µg/g)	Delphinidin-3-glucoside (3. µg/g)	Abdel-Aal et al., 2006; Sharma et al., 2020; Jin et al., 2022; Xiaodan et al., 2020
	Cyanidin (166.2 µg/g)	Cyanidin-3-glucoside (20.5 µg/g)	Peonidin-3-glucoside (162.1 µg/g)	Petunidin-3-glucoside (1.5 µg/g)	
	Peonidin (85.9 µg/g)	Delphinidin-3-glucoside (25.6 µg/g)	Cyanidin diglucoside (71.8 µg/g)	Cyanidin-3-glucoside (1.3 µg/g)	
		Cyanidin-3-rutinoside (11.1 µg/g)	Cyanidin-3-rutinoside (19.9 µg/g)	Delphinidin (1.1 µg/g)	
		Petunidin-3-glucoside (2.3 µg/g)			
		Pelargonidin-3-glucoside (2.1 µg/g)			

for evaluating isogenic lines for TAC content regulatory loci it was found that phenolic content and antioxidant activities in purple-grained lines were not significantly higher than in red-coloured lines (Morgounov et al., 2020). Moreover, the higher protein content or mineral content in pigmented grains was often ascribed to a negative correlation with grain yield (Gaikwad et al., 2020; Kaya & Akcura, 2014; Shah & Wu, 2019).

The use of pigmented cereals in brewing is a result of consumer's demand for a diet supplemented with naturally coloured molecules with various health-promoting activities contrasting obesity, type-2-diabetes, cardiovascular disease, and cancer (Gupta et al., 2021).

The anthocyanin profiles of pigmented grains of five cereal species are shown in Table 2. Based on the literature data, cyanidin-3-glucoside was found to be the most common anthocyanin across all pigmented cereal grains, with rice having the highest content, especially in purple grains (4172 µg/g) and in black grains (2013 µg/g), followed by purple corn (298.9 µg/g) and purple barley (96.9 µg/g). Compared to other cereals, pigmented wheats have much lower contents of all anthocyanins (Table 2). Among other pigments, cyanidin-3-glucoside and pelargonidin are particularly abundant in black corn (969.7 µg/g), whereas cyanidin-3-glucoside is highly present in black rice grains (2013.0 µg/g).

So far, recent literature studies used malt from pigmented rice and corn for brewing (Pozzada dos Santos et al., 2020; Romero-Medina et al., 2020). Based on these, beers improved their composition in antioxidants and other bioactive components (Pozzada dos Santos et al., 2020). It came out that the pigment molecules also provided a typical 'amber-red-cooper' colour to the beers, as well as unique sensory attributes (Romero-Medina et al., 2020). Traditionally, corn was largely used in Mexico to make beer alike beverages, but recently several types of new pigmented corn malt beer were produced (Flores-Calderón et al., 2017).

Purple wheat malt was also used in different ratios with barley malt to widen beer style, flavour, aroma, phenolic content, and antioxidant activity (Dostalova et al., 2015; Li et al., 2007). As a result, the antioxidant activity and total phenolic content increased in the new beer types, even if a large quote of the anthocyanins present in the raw materials went lost during the various steps of beer brewing (Li et al., 2007).

Fig. 3 shows the actual gain of total polyphenols, total anthocyanins, and antioxidant activity of pigmented cereal beers and legume beers as compared with traditional barley malt/hop beers and wine, one of the most noteworthy beverages rich in polyphenols (Flores-Calderon et al., 2017; Li et al., 2007; Luneia et al., 2018; Radonjić et al., 2020; Romero-Medina et al., 2020; Trummer et al., 2021). Even though the

polyphenol content of traditional beers and pigmented wheat-based beers was similar, the enrichment of specific cereal anthocyanin components in the latter had the effect to increase the antioxidant properties of pigmented cereals beers making them nearer to those of red wine.

#### 4. New trends in beer brewing using legume grains as adjuncts

The use of legume grains as malt adjuncts in brewing can contribute to widen the beer market and to meet the consumer demand for new flavours, aromas, and low-alcoholic beers and reduced gluten content. Indeed, legume beers have been produced since the eighteenth century when they were made from a mixture of peas, beans and oats (Markham, 1986). Nowadays, different pulses are used in brewing beers intended for niche markets, especially in Japan and in Europe (Luneia et al., 2018), and are preferred to cereals as malt adjuncts also because of their sustainability and significance in the agro-biodiversity of rural areas (Fiocchetti et al., 2009).

From a nutritional perspective, legume grains provide a cheap source of dietary proteins and are rich in minerals (i.e. potassium, iron and zinc), vitamins, and polyphenols, especially flavonoids, isoflavones, phenolic acids and tannins, which overall have health-promoting effects (Ferreira et al., 2021). Overall, it is estimated that around 85–90% of the global beer production uses adjuncts (Cadenas et al., 2021; Trummer et al., 2021). Nevertheless, while the number of breweries using legumes and other adjuncts is growing, relatively low is the number of research articles investigating on the quality of the new beer types. In the past, the use of peas was preferred to that of other legumes, being peas associated with beer viscosity and good flavour (Markham, 1986). More recently, malted lentils, faba beans flour, and no malted chickling and lentil grains were used as beer adjuncts and beer quality was improved with respect to protein, mineral and polyphenol composition (Black et al., 2019; Luneia et al., 2018; Trummer et al., 2021).

Typically, a low protein content is required to raw materials used for brewing; yet, proteins confer important sensory features to beers such as foam firmness, haze, texture, stability and colour. The partial or total replacement of barley with legume grains is important to reduce the content of gliadin-like amino acid epitopes that can induce inflammatory responses in celiac subjects (Spada et al., 2020). In fact, the adjunction of lentil malt is able to significantly reduce the content of celiac disease epitopes in beers (Trummer et al., 2021). Moreover, an increase of potassium and magnesium and of polyphenols was gained in

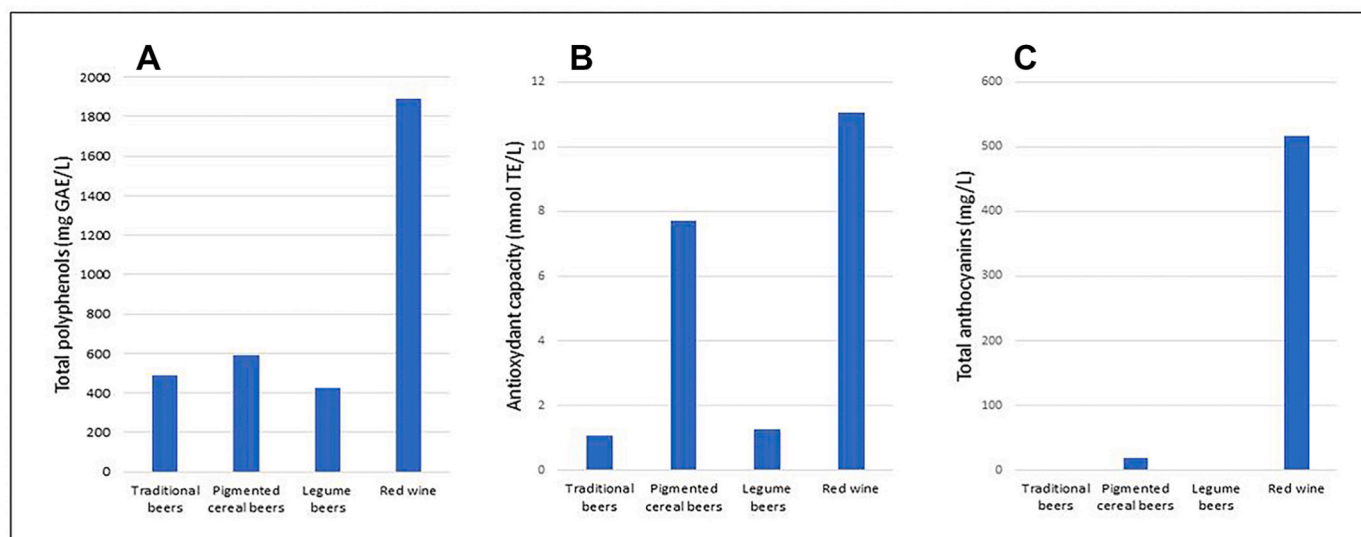


Fig. 3. Polyphenol content (A), antioxidant activity (B) and anthocyanin (C) content of traditional barley malt/hop beers, pigmented cereal-based beers, legume beers and red wine (average contents from Radonjić et al., 2020; Li et al., 2007; Flores-Calderon et al., 2017; Romero-Medina et al., 2020; Trummer et al., 2021; Luneia et al., 2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

beers from no-malt lentil and chickling adjuncts as compared to barley/hop beers (Luneia et al., 2018). Another advantage of legume beers is the lower alcohol percentage compared to traditional beers without affecting sensorial quality, taste and acceptability of final products (Black et al., 2019). Nevertheless, the malts obtained from legume seeds may have some technological features inferior to those of barley malts (Gasiński et al., 2021). Moreover, the addition of lentil malt can result in less fruity beers (Trummer et al., 2021). The Fig. 3 shows the polyphenol content and antioxidant activity of legume beers, comparing them to those of pigmented beers, traditional barley malt/hop beers, and wine. Though the use of legumes seems to do not have a positive effect on these traits, it was shown that legume adjuncts improve the content of specific isoflavones (i.e. genistin and daidzin) that, besides having antioxidant and anti-inflammatory properties, contribute to preventing cancer, cardiovascular, postmenopausal, and immune diseases (Luneia et al., 2018).

### 5. Healthy and nutritional characteristics of pigmented cereals and legume's beers

Beer is rich in bioactive compounds coming from traditional ingredients (i.e. barley, hop), but also from special ingredients such as pigmented cereals and legumes, which can affect the nutritional composition of the final product. As in other fermented beverages, such as wine, the composition of beer includes an important non-ethanolic fraction represented by bioactive substances, including phenolic compounds, endowed with multiple beneficial effects on human health and disease prevention (Calabriso et al., 2020). Several clinical trials showed that a moderate consumption of beer could mimic some of the previously reported health properties of red wine, such as the antioxidant and anti-inflammatory effects of polyphenols, also reducing the risk of cardiovascular diseases (Chiva-Blanch et al., 2015). It was suggested that the polyphenolic content of beer improves the lipid profile and reduces inflammatory biomarkers related to atherosclerotic process. Nutritional studies also showed a protective effect of polyphenols on the cardiovascular system, reducing the risk for ischemic stroke, congestive heart failure, peripheral arteriopathy, and coronary heart disease, thus strengthening the idea that also beer phenolic compounds are allies in the prevention of chronic and degenerative diseases (De Gaetano et al., 2016).

In the field of beer brewing, the use of raw materials rich in bioactive compounds, such as pigmented whole grains and legumes improve the quantity and quality of phenolic compounds (Bassolino et al., 2022; Black et al., 2019; Radonjić et al., 2020; Trummer et al., 2021).

Anthocyanins have demonstrated antioxidant potential both in *in vitro* and *in vivo* studies, and the consumption of foods and beverages high in anthocyanins has been linked to lower risks of chronic diseases (Francavilla & Joye, 2020). In animal model, the integration of anthocyanin-rich food decreases atherosclerotic plaques in rabbits and increases the endogenous antioxidant status in apolipoprotein E-deficient mice. Moreover, an anti-ageing and neuroprotective effect of anthocyanin-rich diet has been suggested. In fact, several *in vivo* studies indicate that food supplementation with pigmented cereals, rich in anthocyanins, may contribute to prevent metabolic syndrome by ameliorating the negative effects of high-fat or high-sugar diets (Gonçalves et al., 2021). Beneficial effects in decreasing cholesterol metabolism, and preventing hypertriglyceridemia, weight gain and insulin resistance with an improvement on gut microbiota dysbiosis were also reported (Bassolino et al., 2022).

However, as for other products such as bread or pasta (Bartl et al., 2015; Ficco et al., 2014) a significant reduction of the anthocyanins is lost during the brewing process (dos Santos et al., 2020; Li et al., 2007). In particular, Spessoto et al. (2020) have observed a lost (equivalent to the 95% of the initial amount detected in the cob) of the total anthocyanin content during the brewing of the "Chicha Morada" beer, obtained of the purple corn.

While a large body of evidence show the complementary effect of cereals and pulses in protecting against inflammatory diseases (Awika et al., 2018), further studies are needed to assess the bioavailability and synergistic effect of cereals and pulses polyphenols in beers.

### 6. Sensory properties of pigmented cereals and pulses beers

The sensory profile of beer is determined by a wide diversity of volatile and non-volatile compounds that may depend upon the used raw materials (Flores-Calderón et al., 2017). Due to the growing interest of consumers in beverages with a high health value and innovative sensory notes, pigmented cereals and legumes were also considered for brewing. When red and blue corn were employed for brewing, they provided peculiar sensory notes to the beers (Romero-Medina et al., 2020). These were especially due to aldehydes, alcohols, ketones and esters, among the most representative. While the aldehydes (i.e. 3-methyl-butanal, 2-methyl-butanal, hexanal, (E)-2-hexenal, heptanal, benzaldehyde, benzenacetaldehyde, (E)-2-octenal, and (E)-2-nonenal) were responsible for vegetable-like aroma, the esters were associated with fruity and floral notes (Romero-Medina et al., 2020; Thompson-Witrick & Pitts, 2020). Indeed, no significant differences were found between traditional beers and pigmented corn-based beers with respect to alcohols, such as 1-pentanol, 1-hexanol, 1-octen-3-ol, and 2-ethyl-1-hexanol. Conversely, the volatile phenolic profile is largely influenced by the use of corn (Seitz & Ram, 2000). It was found that 2-methoxy-phenol and 2-methoxy-4-vinylphenol, associated with a smoky odour, were both identified in pigmented corn based beers (Romero-Medina et al., 2020). Other studies considered the effect of purple, blue, yellow, and white wheat grains on the overall sensorial quality of beers showing that purple wheat was the best pigmented cereals, especially when it was used in combination with barley malt (Li et al., 2007).

When legumes were used as malt adjuvants, they provided a slight peach aroma to the beers (Trummer et al., 2021). This evidence was confirmed after the evaluation of craft beer fortification with red lentil hydrolysate (Canonico et al., 2022). The addition of this adjuvant resulted in improved flavour complexity due to an increase of alcohols, esters, and carbonyl and sulphur compounds.

The acceptance of beer among consumers can be influenced by the content and composition of polyphenols due to their influence on the taste of bitterness and astringency. However, the increase of polyphenols due to the use of hop, or of other raw materials rich in polyphenols, such as pigmented cereals and legumes, may enhance the formation of protein-polyphenol hazes, which can limit beer's shelf life (Aron & Shellhammer, 2010). However, due to either the positive or negative effects of polyphenols on the taste and aroma of beers, their role in determining the beer sensorial quality is still debated (Aron & Shellhammer, 2010).

### 7. Yeast contribution to beer's aroma profile of pigmented cereals and legume grains beers

Several yeast species hold a key role in the brewing process, since during wort fermentation they either metabolizes the sugar and synthesize several molecules that delineate the peculiar sensorial features of the beverage. Different *Saccharomyces* species are in charge of the alcoholic fermentation of a large variety of foodstuffs enabling the production of fermented foods and beverages. However, the capacity to catabolize maltose and maltotriose is not common, thus limiting the brewing attitude only to a limited number of species. Moreover, the strain-specific ability to produce secondary fermentative, i.e. esters, aldehydes, organic acids and higher alcohols, is essential for outlining the aroma and flavour of the final products (De Simone et al., 2021). Brewing yeast species cluster into two main groups. The former is composed of *Saccharomyces cerevisiae* strains that ferment at higher temperatures (16–22 °C) and aggregate at the top of the vessel when fermentation ended. The latter includes *S. pastorianus* (syn.

*S. carlsbergensis*), which ferments at lower temperatures (6–16 °C) and lay at the bottom of the container at the end of the process (Petruzzi et al., 2016). The beer produced by spontaneous fermentation has a typical sour taste due to from the contemporary fermentation by different yeast (*Saccharomyces*, *Dekkera/Brettanomyces*) and lactic acid bacteria (LAB; *Lactobacillus* and *Pediococcus*) species (Spitaels et al., 2014). In the traditional Belgian lambic style, a microbial consortium composed of more than 2000 strains has been described (Spitaels et al., 2014).

Numerous investigations are nowadays aiming at the employment of the microbial biodiversity valuable as novel perspective for the beer industry, such as *Saccharomyces cerevisiae* strains isolated from other fermented food and beverage hybrids of the *Saccharomyces* genus and non-*Saccharomyces* species. The request for innovative starter cultures for beer production is nowadays increasing with the consequent increase in the study of microbial biodiversity useful for brewing (Cubillos et al., 2019; Iattici et al., 2020).

The selective procedures are directed to identify strains denoted with properties of pro-technological interest such as ethanol tolerance, maltose and maltotriose utilization and production of desired volatile compounds (De Simone et al., 2021).

Numerous commercial *S. cerevisiae* starters have been utilized for the fermentation of wort derived from pigmented cereal. Romero-Medina et al. (2020) have assessed the sensory profile of craft beers produced from pigmented corn, by using the US-05 starter strain (Fermentis, France). The Authors were able to detect several sensorial notes, such as dried fruits and chili, fermented fruits, cooked vegetables, bread and tortillas. Moreover, among a hundred of volatile by-products recognized by head space-solid phase micro-extraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS), phenols and terpenes were the most represented groups of molecules.

Several non-*Saccharomyces* species are received considerable commercial and technological interest due to their specific pro-technological abilities and the positive effect on the sensorial properties of alcoholic beverages (Tufariello et al., 2021). Different genera have been investigated for their contributions to brewing protein-enriched beers. A recent study has assessed the performance of selected non-*Saccharomyces* strains in the production of craft beer from wort enriched with lentils extract (Canonico et al., 2021). The Authors showed that two strains of *Lachancea thermotolerans* and *Kazachstania unispora* contributed to increase the concentration of many aromatic compounds such as isoamyl acetate, ethyl acetate and higher alcohols, thus enhancing the sensorial profile and the aromatic attributes of the produced beers.

Craft beers produced by the spontaneous fermentation of pigmented sorghum are the most popular traditional alcoholic beverages in Africa. They are identified by several names (Dolo, Doro or Chibuku, Ikikage or Amarwa and Merissa) depending on the region or ethnic group (Sawadogo-Lingani et al., 2021). Strains belonging to *S. cerevisiae* species were the dominant yeast species involved in the alcoholic fermentation, with a biodiversity at strain level. Lactic acid bacteria involved in the brewing process belong to the genera of *Limosilactobacillus*, *Lentilactobacillus*, *Pediococcus*, *Leuconostoc*, *Lactococcus*, *Streptococcus*, and *Enterococcus*. The different microbial consortia associated with spontaneous fermentation processes determine the organoleptic, aromatic, and sensory characteristics that characterize the different typical beers (Sawadogo-Lingani et al., 2021).

## 8. Quality control and authentication of traditional and special beers

Improving beer for functional and healthy quality requires a greater attention to some safety issues to avoid the potential occurrence of dangerous contaminants (physical, chemical, or microbiological) and preserve consumers' health (Ciont et al., 2022; Laddomada et al., 2014). Especially craft beers can be more prone to spoilage compared to beers coming from large-scale industrial breweries. So far, certifying the

quality and safety of novel functional craft beers would increase the trust of consumers. Another urgent issue for brewers is to protect craft beers with novel health-promoting properties, tastes and aromas from adulterations and frauds.

Among the quality control procedures that are used in brewing, capillary electrophoresis, mass spectrometry, and MALDI-TOF-MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) are specific for proteome characterization, or to detect the occurrence of microorganisms involved in beer spoilage (Anderson et al., 2019; Condina et al., 2019), whereas ICP-MS (Inductively Coupled Plasma Mass Spectrometry) is commonly used to detect metal contaminants such as arsenic, cadmium, and lead (Anderson et al., 2019).

For quality control, ultraviolet visible (UV) and infrared (IR) spectroscopy are used, supported by more sophisticated approaches based on gas and liquid chromatographic techniques (GC, HPLC) to obtain analytical profiling of the content and composition of fatty acids, essential oils, aroma components, and individual phenolic components with antioxidant activity (Anderson et al., 2019). Moreover, electron spin resonance (ESR) spectroscopy was applied to evaluate potential antioxidants in beer (Andersen et al., 2000) and differential scanning calorimetry (DSC) demonstrated its potential in estimating quality, nutritional, and thermophysical properties of foods and beverages during processing and storage (Laddomada et al., 2013; Parniakov et al., 2018; Pasqualone et al., 2018). In addition, high throughput metabolic profiling based on the potential of high-resolution Nuclear Magnetic Resonance (NMR) Spectroscopy and Mass Spectrometry (MS), when combined with multivariate statistical analysis approach, are among the most convenient to certify the authenticity of special beers, crafts beers, and new functional beers (Cavallini et al., 2021; Vasas et al., 2021). Indeed, the complementary potential of NMR and headspace SPME GC was demonstrated for the identification and quantification of key flavour and aroma compounds of beers (Johnson et al., 2017).

## 9. Conclusions

Functional beers have become a new trend in beer brewing. The use of pigmented cereals for brewing was described in recent literature studies showing the gain in phenolic components and antioxidant activity with respect to traditional beers. In addition, on-going works on the use of pulses as malt adjuncts are demonstrating their potential to improve beer quality for flavour and specific health-promoting components, without affecting the consumer's acceptance of the new beer types. However, further investigations are needed to test the influence of different technological processes, such as malting, milling, boiling, cooling and fermentation on anthocyanins and total polyphenols contents, and antioxidant activity. In addition, more studies are needed to test the potential of spontaneous fermentation processes on the organoleptic, aromatic, and sensory characteristics of pigmented cereals and pulses beers. Finally, further *in vivo* investigations have to be set up to evaluate the effect of the consumption of these new beers on human health, particularly investigating on the bioavailability and synergistic effects of their polyphenol components.

## CRedit authorship contribution statement

**Giuseppe Romano:** Writing – original draft, Visualization. **Maria Tufariello:** Writing – original draft. **Nadia Calabriso:** Writing – original draft. **Laura Del Coco:** Writing – original draft. **Francesco P. Fanizzi:** Writing – original draft. **Antonio Blanco:** Conceptualization, Writing – original draft, Visualization. **Maria A. Carluccio:** Writing – original draft. **Francesco Grieco:** Conceptualization, Writing – original draft, Writing – review & editing. **Barbara Laddomada:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

All authors declare no conflict of interest.

## Data availability

No data was used for the research described in the article.

## Acknowledgments:

This research was partially funded by PRIMA Section 1 2020 Agro-food Value ChainIA Topic: 1.3.1–2020 (IA), MEDWHEALTH, project grant no. 2034, and by the project “Birra: dal campo al boccale - BE<sup>2</sup>R” (P.S.R. Puglia 2014/2020 -Misura 16). The Authors are grateful to Domenico Genchi (CNR-ISPA) for his skilled technical support and Pasquale Del Vecchio (CNR-ISPA) for the data stewardship supplied.

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