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REVIEW ARTICLES

Occurrence of fumonisins and strategies for biocontrol in beer production: a systematic review

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Highlights

- Conventional food processing methods are not able to eliminate fumonisins, and biological control methods are the most effective, as they reduce or elimite them
- The main biological control strategies for fumonisins reduction during beer processing are the adsorption and enzymatic biodegradation methods
- A major problem is the risk of exposure to FBs through consumption of contaminated beer, as some studies have shown that contamination by FBs can persist throughout the beer production process, specifically when the initial concentration is high
- The occurrence of FBs fumonisins in beer results from the use of contaminated raw material, mainly corn-based adjuncts, but also barley

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KEYWORDS
Biological manipulation;
Biological control;
Corn;
Barley;
Beers.

Abstract: Beer is one of the most consumed alcoholic beverages in the world and is basically made up of water, barley malt, hops and yeast other components that also be added in production such as adjuncts. Corn is among the most used adjuncts and considered a viable and affordable grain to partially replace barley. However, there is a constant concern about the occurrence of mycotoxins and subsequent contamination in beer processing. Corn can be contaminated by a type of mycotoxin called fumonisins, produced by the fungus *Fusarium verticillioides*. The relationship between the detection of fumonisins and the use of corn-based adjuncts in beer processing has been described for over 25 years. However, the occurrence and effect on beer processing are less reported in the literature when compared to scientific publications on the relationship between the fungus *F. graminearum* and its mycotoxins. Given this scenario, the objective of this revision was to develop a *Methodi Ordinatio* systematic literature review on three subjects: fumonisins occurrence in beer, the contamination effect by *F. verticillioides* and fumonisins on beer processing and viable biocontrol methods to improve this problem. In total, 22 articles on the occurrence of fumonisins in beer were selected, which showed that countries on the African continent are the ones with the highest levels of mycotoxins contamination. In addition, 17 papers were selected to discuss the effect of contamination by *F. verticillioides* and fumonisins on beer processing. Together, these works verified the presence of fumonisins in the raw material and in the final product after processing, demonstrating that more measures are needed to restrict the development of fumonisin-producing fungi. Finally, 21 papers were selected on viable biocontrol methods to improve beer processing. Specifically, it has been described that conventional food processing methods are not able to eliminate fumonisins, and biological control methods are more effective as they reduce or eliminate them. Such methods involve physicochemical processes such as adsorption and enzymatic biodegradation.

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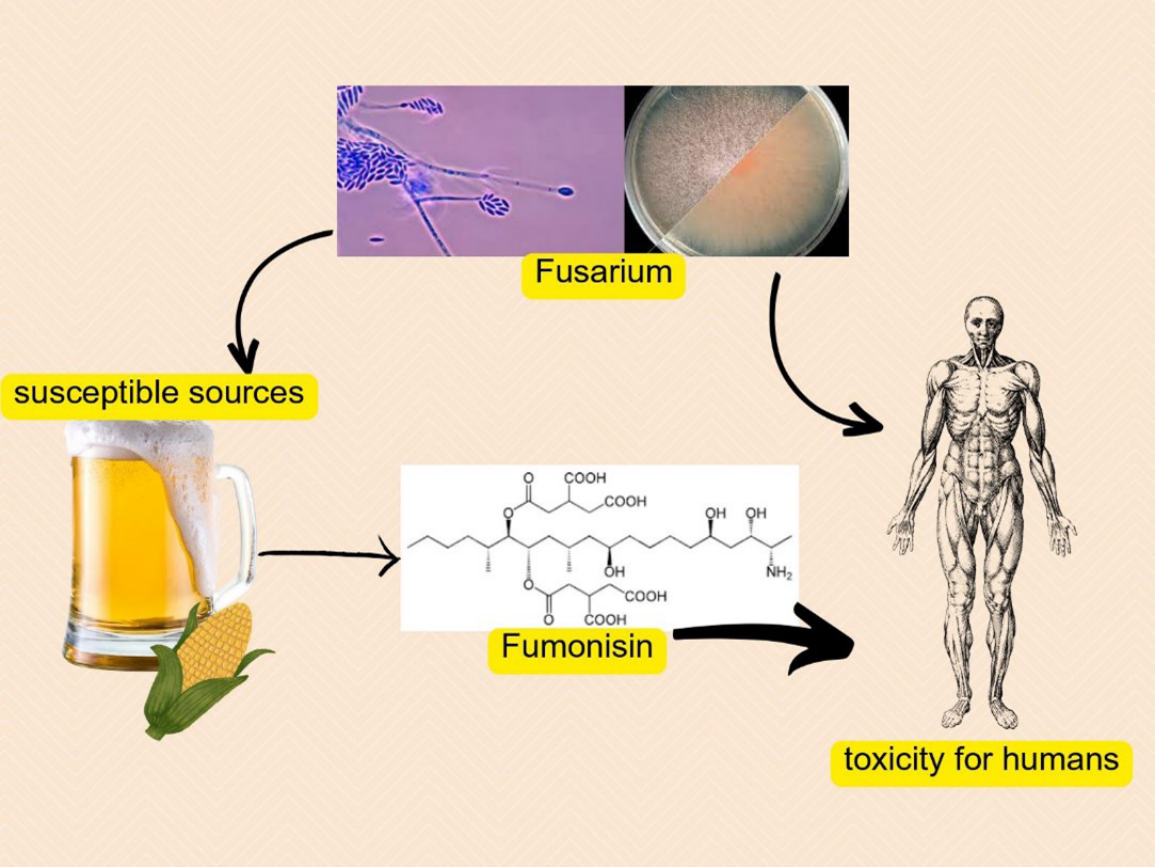
Graphical Abstract



Introduction

Beer is one of the most consumed alcoholic beverages in the world and basically consists of water, barley malt and hops, other components can also be added in production, such as adjuncts. Corn is among the most used adjuncts in brewing, a viable and affordable grain to partially replace barley (Sleiman et al., 2010; D'Avila et al., 2012).

Grains and cereals are the main raw materials in beer production, and there is a constant concern about the occurrence of mycotoxins in their processing (Gonçalves et al., 2012). Most studies on the occurrence of mycotoxins in beer focus on those produced by *Fusarium graminearum*, such as deoxynivalenol, nivalenol, T-2, HT-2, diacetoxyscirpenol and zearalenone, present in barley (Gonçalves et al., 2012). However, corn can be contaminated by other types of mycotoxins, such as fumonisins (FBs), produced by *F. verticillioides*.

The relationship between FBs detection and the use of corn-based adjuncts in beer processing has been described for over 25 years (Scott et al., 1995). However, the occurrence and effect on beer processing are less reported in the literature when compared to scientific publications on the relationship between the *F. graminearum* and its mycotoxins.

Given this scenario, the objective of this article is to develop a systematic review of the literature on three subjects: (a) the occurrence of FBs in beer, (b) the effect of contamination by *F. verticillioides* and FBs on beer processing and (c) viable biocontrol methods to improve this problem.

Material and methods

This study developed a Methodi Ordinatio systematic review (Pagani et al., 2015), which uses the Index Ordinatio (InOrdinatio) equation to choose and classify works according to their scientific relevance. Steps and eligibility criteria are detailed below. The first step was to determine the research intent, summarized in the following problem question: "How do FBs affect beer processing, and What are the most effective biological controls in this process?". Subsequently, the keywords were determined. Four keywords were selected regarding the occurrence of FBs and beer processing: "fumonisin", "beer", "malt" and "brewing", with parentheses and an asterisk and using the Booleans "AND" and "OR". For the FBs biocontrol, the keywords used: "fumonisin", "biological control", "degrad", "degradation", "biodegrad", "remove", "adsorb" and "adsorbent" also using parentheses, asterisk and the boolean "AND".

The databases chosen for the search were Science Direct, Scopus and Web of Science and the study period included the literature published between 1993 and 2021. The initial search resulted in a total of 271 papers on the occurrence and processing of FBs and 879 papers on the biocontrol of these mycotoxins in beer production.

Subsequently, a filtering was developed in which the following criteria were adopted: (a) exclusion of duplicate articles, for which Mendeley software, version 2.61.1 (Elsevier, London, England) was used; (b) the remaining

articles were transferred to the software JabRef, where a new review was carried out to verify the titles and abstracts that fit with the theme of the work; (c) articles unrelated to the topic were excluded; and (d) then, a full reading of the remaining articles was developed to confirm alignment with the subject.

Data from selected articles were exported to a Microsoft Excel® spreadsheet.

The articles were separated into new spreadsheets and the InOrdinatio equation was used to classify the articles in order of relevance, according to the metric of the year in which it was published, number of citations and impact factor (Journal Citations Reports - JCR). It is noteworthy that the articles choice followed criteria imposed by the researchers, which may result, in a different way, in the choice of other relevant articles (Figure 1).

In total, 22 articles were selected on the occurrence of FBs in beer, 17 on the effect of contamination by F. verticillioides and FBs on beer processing and 21 works on viable biocontrol methods to improve this processing (Supplementary Material 1, 2 and 3, respectively). In addition, VOSviewer software, version 1.6.15 (van Eck and Waltman, Leiden, Netherlands) (Van Eck & Waltman, 2010) was used to create visual maps, which illustrate author co-citations, journal co-citations and keyword co-occurrence network.

Results

Occurrence of fumonisins in beer

Among the studies in which FBs were detected, 8 articles identified samples originating from commercial beers from countries such as Spain, Ireland, Brazil, and Italy (Table 1).

In addition, five studies did not detect FBs in commercial beers. Among these works, 12 samples were from South Korea (Seo et al., 2009), 24 from Japan (Tamura et al., 2011), 25 from Ireland (Puangkham et al., 2017), 34 from Tunisia (Juan et al., 2017) and 100 from Thailand (Rubert et al., 2011). On the other hand, Table 2 presents data related to FBs specifically detected in beer samples produced with corn adjuncts.

Fusarium sp. and fumonisins in beer processing

Several studies have analyzed the contamination with fumonisin 1 (FB_1) in wort, grains, and beers. On this subject, 17 studies were selected which have investigated the possible FBs transfer from the malted grain to beer (Supplementary Material 2), and Table 3 presents 6 studies that used corn-based adjuncts and sorghum beer exclusively.

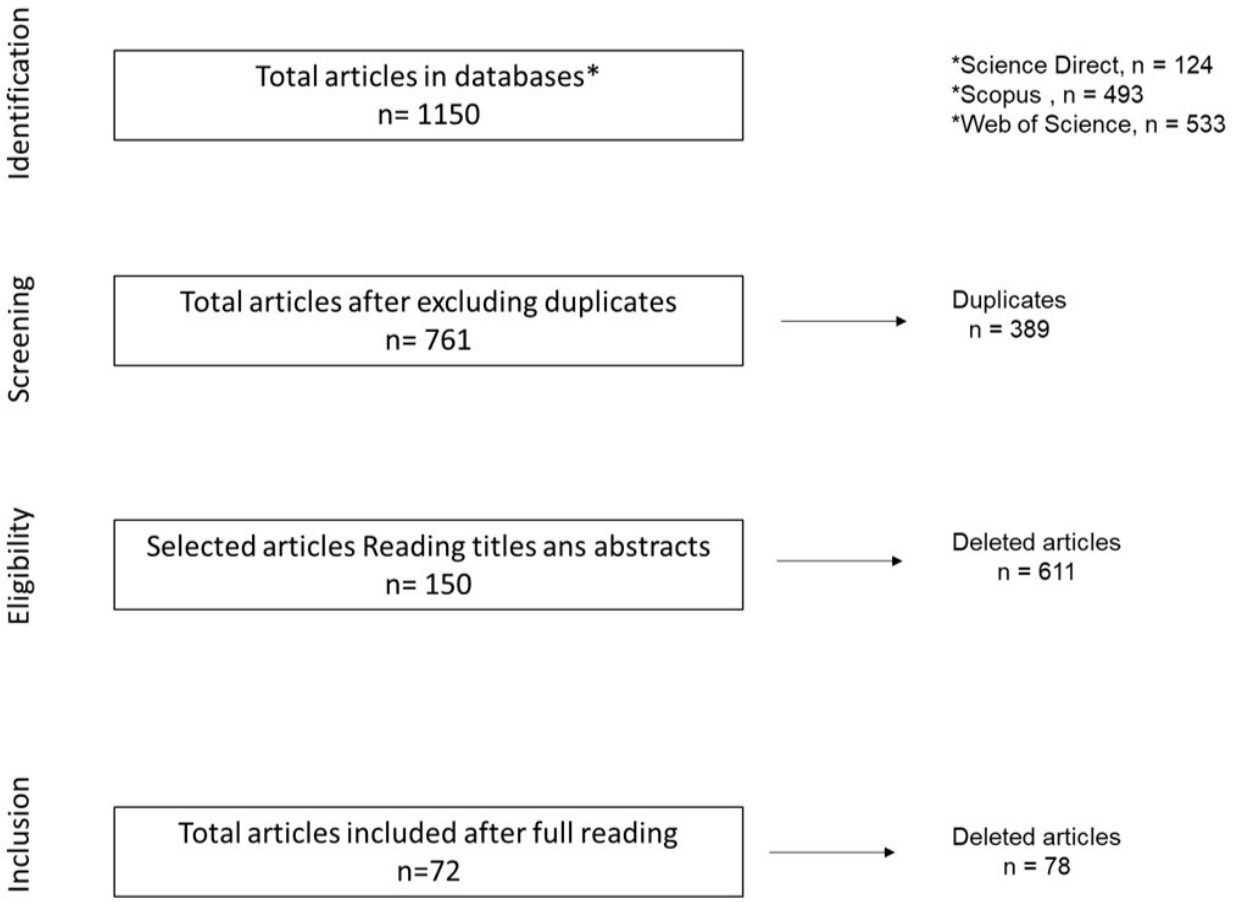


Figure 1. Flowchart of the literature review process.

Table 1. Fumonisins in samples of commercial beer from different countries.

Country of origin	Method	N	positive N (FB)	FBs (concentration range or average)	Reference
Spain	HPLC	5	5	FB_1 (200 µg/kg) FB_2 (100 µg/kg)	Cano-Sancho et al. (2011)
		216 72 composite samples	64 (FB_1 + FB_2)	FB_1 + FB_2 (average de 36.9+20.1 ug/kg)	Cano-Sancho et al. (2012)
Spain	UHPLC-(ESI)-MS/MS	10	10 (FB_1)	FB_1 (1.3 a 13 µg/kg)	Beltrán et al. (2013)
Ireland	HPLC-QqQ-MS/MS	49	10 (FB_1 + FB_2)	FB_1 (71.2 a 127 ng. mL) FB_2 (71 a 96.1 ng. mL)	Rubert et al. (2013)
Brazil	HPLC	58	25 (FB_1)	FB_1 (1.2 a 40 ng/mL)	Kawashima et al. (2007)
		53	8 (FB_1)	FB_1 (29 a 285 ng/g)	Piacentini et al. (2015a)
		114	56 (FB_1)	FB_1 (201.70 a 1568.62 µg/L)	Piacentini et al. (2017)
Italy	LC-MS/MS	75	16 (FB_1) e 2 (FB_2)	FB_1 (0.6 a 12.3 ng/mL) FB_2 (0.7 ng/mL)	Campone et al. (2020)

Abbreviations: Fumonisin FB (FB); Fumonisin FB1 (FB1); Fumonisin FB2 (FB2).

Table 2. Fumonisins in beer samples with corn adjunct.

Country of origin	Method	N	positive N (FB)	FBs (concentration range or average)	Reference
Canada and other contries	HPLC	41	4 (FB_1)	FB_1 (2 a 59 ng/mL)	Scott et al. (1995)
USA and other contries	HPLC	29	25 (FB_1) 12 (FB_2)	FB_1+FB_2 (<0.3 a 13.5 ng/mL)	Hlywka & Bullerman (1999)
	HPLC	18	18 (FB_1)	FB_1 (38 a 1066 ng/mL) FB_1 + FB_2 + FB_3 (43 a 1329 ng/mL)	Shephard et al. (2005)
South Africa	LC-MS	32	17 (FB_1), 10 (FB_2), 2 (FB_3),	FB_1 (151 µg/L) FB_2 (96 µg/L) FB_3 (36 µg/L) FB_1 e FB_2 (132 µg/L) FB_1 + FB_2 + FB_3 (média de 125 µg/L)	Adekoya et al. (2018)
Japan	HPLC	30	10 (FB_1)	FB_1 (média de 4.7 µg/kg) FB_2 e FB_3 (nd)	Aoyama et al. (2010)
Several countries in Europe	CG/MS e HPLC/MS	33	32 (FB_1) e 19 (FB_2)	FB_1 (<0,1 a 30.3 µg/µl) FB_2 (<0,1 a 3.9 µg/µl)	Bertuzzi et al. (2011)
Cameroon	LC-MS	14	14 (FB_1) e 1 (FB_6)	FB_1 (15-741 µg/g) FB_2 (0.6 e 127 µg/g) FB_3 (0.7 a 100 µg/g) FB_6 (76.13 µg/g)	Abia et al. (2013)
Malawi (Africa)	LC-MS	9	9 (FB_1) 8 (FB_2) e 6 (FB_3)	FB_1 (1522+1192 µg/kg) FB_2 (251+206 µg/kg) FB_3 (229+161 µg/kg) FB_1 + FB_2 (1745+1294 µg/kg) FB_1 + FB_2 + FB_3 (1898+1405 µg/kg)	Matumba et al. (2014)

Abbreviations: Fumonisin FB (FB); Fumonisin FB_1 (FB_1); Fumonisin FB_2 (FB_2); Fumonisin FB_3 (FB_3); Fumonisin FB_6 (FB_6).

Biocontrol for fumonisins reduction in beer processing

To identify the knowledge related to biocontrol methods for the reduction of FBs in beer processing, 21 works were selected using the Methodi Ordinatio method (Supplementary Material 3). However, 17 studies allowed us to recognize that the main biological control strategies in this process involve adsorption methods and enzymatic biodegradation methods (Table 4).

In this case, a total of five articles described the control by adsorption, mainly by lactic acid bacteria, strains of *Lactobacillus* and *S. cerevisiae*. On the other hand, 12 articles presented the enzymatic biodegradation method, which includes the action of microorganisms such as *Fusarium proliferatum*, *Sphingopyxis* sp. MTA144, *Pleurotus eryngii*, *Delftia/Comamonas* group, *Lactobacillus* strains, bacterial consortium SAAS79 (with *Pseudomonas* dominance) and FumD fumonisin esterase (FUMzyme®).

Table 3. Variation in the concentration of FB1 in the raw material and in the final beer production process.

Initial sample	Contamination	Final Product	[FB_1] inicial	[FB_1] final	Reference
Wort	Artificial	Beer	0.95 µg/mL	0.02 a 0.26 µg/mL	Scott et al. (1995)
Corn	Natural	Beer	1146 to 3194 µg/kg	37 to 89 µg/l	Pietri et al. (2010)
	Artificial	Beer	201.7 a 1568.62 µg/L	367.47 µg/kg (média)	Piacentini et al. (2017)
	Artificial	Wort	50 a 750 µg/kg	50 to 300 µg/kg	Pascari et al. (2019)
Sorghum	Natural	Beer	47 a 1316 µg/kg	0 µg/kg	Nkwe et al. (2005)
	Artificial	Beer	806 µg/kg	20 µg/kg	Chilaka et al. (2018)

Abbreviations: Fumonisin FB (FB); Fumonisin FB1 (FB1).

Table 4. Main strategies for the fumonisins biocontrol in beer production.

Biocontrol	Microrganism	FB Studied	Reference
Adsorption	Lactic acid bacteria	FB_1	Mokoena et al. (2005)
	Lactic acid bacteria	FB_1 e FB_2	Niderkorn et al. (2009)
	Saccharomyces cerevisiae CECT 1891 and *Lactobacillus acidophilus* 24	FB_1	Pizzolitto et al. (2012)
	Saccharomyces cerevisiae	FB_1	Armando et al. (2013)
	Strain of *Lactobacillus*	FB_1 e FB_2	Zhao et al. (2016)
Enzimatic	*Fusarium proliferatum*	FB_1	Keller & Sullivan (1998)
	Delftia/Comamonas Group	FB_1	Benedetti et al. (2006)
	Sphingomonas sp. MTA144	FB_1	Heinl et al.(2009)
	Sphingopyxis sp. MTA144	FB_1	Heinl et al. (2010)
	Sphingopyxis sp. MTA144	FB_1	Hartinger et al. (2010)
	Sphingopyxis sp. MTA144	FB_1	Hartinger et al. (2011)
	Sphingopyxis sp. MTA144	FB_1	Li et al. (2021)
	Pleurotus eryngii	FB_1	Haidukowski et al. (2017)
	Strains of *Lactobacillus*	FB_1	Martinez-Tuppia et al. (2017)
	Bacterial consortium SAAS79 (com dominância de *Pseudomonas*)	FB_1	Zhao et al. (2019)
	Fumonisin esterase FumD (FUMzyme®)	FB_1	Alberts et al. (2019)
	Fumonisin esterase FumD (FUMzyme®)	FB total	Alberts et al. (2021)

Abbreviations: Fumonisin FB (FB); Fumonisin FB_1 (FB_1); Fumonisin FB_2 (FB_2).

Discussion

Barley malt is the main source of fermentable sugar for beer production. However, due to the low cost of some adjuncts and/or the local availability, desirable sensory properties, adjuncts are commonly used in the brewing industry in the mashing stage. Among the main adjuncts, corn-based products in the form of syrup or gritz, rice, unmalted barley, wheat, and sorghum stand out (Oliveira, 2011; Piacentini et al., 2017). These grains and cereals are susceptible to fungal contamination, being of particular attention to the work that showed contamination caused by the fungus *F. verticillioides*, the main producer of FBs mycotoxins. This is a non-obligate parasitic fungus, potential mycotoxigenic, commonly occurring in maize crops, causing root, stalk and ear rot and spoilage of stored grains. This fungus is commonly found in corn samples and corn-based products, in addition to being able to produce several mycotoxins, the most prominent being FB_1 (Bowers & Munkvold, 2014). This mycotoxin can be introduced into beer, either from contaminated inputs or from adjuncts added during the mashing process.

The results of the present literature review described 8 scientific articles that detected the FBs occurrence in beers around the world (Table 1) and 8 articles which demonstrated the FBs presence in beer samples with corn adjunct (Table 2) from 1993 to 2021. Scott et al. (1995) was the first to report FB_1 contamination in beer, although they detected FB_1 only in 4 samples out of 41 samples analyzed and at relatively low concentrations (>59 ng/mL). Similarly, compared to other countries the detection range for FB_1 and fumonisin 2 (FB_2) respectively ranged from 1.3 to 127 ng/mL and 0.7 to 96.1 ng/mL. Commercial beer samples from Brazil showed an increase in the concentration of FB_1, from 2007 to 2017 (Table 1), a situation that denotes the importance of controls in beer production. On the other hand, among the articles which describe fumonisins present in beer with corn adjunct (Table 2), recurrent contaminations in African countries stand out, a situation which may reveal trends in FB contamination that are important to understand. In this case, the tropical climate of these countries favors the *Fusarium* spp. development (Adekoya et al., 2018; Piacentini et al., 2015a). Another issue refers to control, the lack of laws and stricter measures control in the production of craft beers, aspects which can favor the presence of such contaminants in these products.

In Africa, fermented foods and beverages are traditionally home-made, without adequate controls to ensure safety during production (Matumba et al., 2014). In this sense, the literature argues that the presence of multiple mycotoxins in beer samples produced in African countries is a direct consequence of the poor and uncontrolled processing conditions of the products. In particular, in the malting process, which involves the increase of mycotoxins in the grains, which at the same time interact with a humid environment, conducive to their development (Matumba et al., 2014; Adekoya et al., 2018).

Regarding the presence of *Fusarium* sp. and fumonisins in beer processing, it can be mentioned that beer production processes significantly reduce the concentrations of mycotoxins, but do not eliminate them completely, as can be analyzed by looking the data in Table 3. For example, studies with FB_1 and FB_2 added at various stages of the fermentation process have shown that these mycotoxins can be transferred from contaminated grains to beer (Scott, 1996). Thus, they can be introduced into this beverage if grains and cereals or their contaminated products are used in processing (Boeira et al., 2000; Scott, 1996). In addition to the risk of FBs in the final product, contamination of the raw material can also affect fermentation during beer processing. In relation to brewing yeasts, there is the possibility of adsorption of mycotoxins, thus reducing their concentration in the product or inhibition of the yeast by the toxic effect of the mycotoxin (Pinheiro et al., 2017). About this, a work developed by Scott et al. (1995) analyzed the fermentation effect on FB_1 and FB_2 added to wort. These mycotoxins were added at concentrations of 0.95 µg/ml, and the wort was fermented for up to 8 days by three different *Saccharomyces cerevisiae* strains. The results of this research demonstrated that FB_1 and FB_2 were quite stable in the fermentation process. The estimated losses of FB_1 and FB_2 were 3 to 28% and 9 to 17%, respectively, over the 8 days. This information denotes that the adsorption of FBs by yeast was insignificant, less than 1% for FB1 and 2% for FB2.

In contrast, Kłosowski & Mikulski (2010) described the influence of selected mycotoxins on the main characteristic factors of the corn wort fermentation process, such as alcohol concentration, productivity, yield and energy. Alcoholic fermentation indicators of worts made from raw material with low level of contamination were compared with worts obtained from raw material selectively contaminated with mycotoxins FB_1 (1875 ppb), FB_2 (609 ppb), and FB_3 (195 ppb). The authors described that these FBs did not substantially affect the course of subsequent fermentation stages, the first and main stages of beer fermentation.

At this point, it is important to mention that the analysis carried out in the articles reviewed in this work, demonstrated that the addition of barley contaminated with *F. verticillioides* in the malting phase, the addition of adjuncts contaminated with fungi in the mashing phase and in the fermentation phase, are presented as the three main stages with the highest risk of developing contamination by FBs in beer production. The results of this review also show that the maceration phase substantially reduces the FBs concentration. In this phase, the grains intended for the beer production are placed in water, raising the moisture content so that they can germinate. Due to this action, FBs that may be present in these grains are diluted and therefore eliminated (Maenetje & Dutton, 2007). However, despite this contamination remaining until the final product, with lower values, the studies reveal that the raw materials and final products complied with the limits set by the European Commission Regulations, and other national and international regulations. Therefore, the contribution of low level intake of FBs increase the risk for the consumer. Another objective of this work was to investigate possible biological control methods which can be used during beer processing. Among the main strategies, adsorption methods (substances with functional groups which could interact with mycotoxins, adsorbing them and reducing their absorption in the consumer's gastrointestinal tract) and enzymatic biodegradation methods (Table 4).

FBs adsorption mechanisms have been extensively studied with lactic acid bacteria. These microorganisms are capable of binding to FBs in a dependent manner of pH, species, bacterial density, and analogue (FB_2>FB_1), under *in vitro* conditions.

In this case, the study by Niderkorn et al. (2009) discover that the FBs binding sites are peptidoglycans (PGs) or compounds strongly associated with them. The structure of PGs varies mainly in the amino acid at position 3 (AA3) of the peptide bridge and in the cross-linking amino acids. This difference could explain their differential efficiency in binding BAL to FBs. Such binding of FBs is fast and particularly effective in acidic conditions, forming a stable complex in the pH range present in the gastrointestinal tract (Niderkorn et al., 2007). On the other hand, research involving fumonisins degradation advanced with the discovery of new strains with enzymatic apparatus capable of degrading the toxin, but also with the advancement of analysis methods. Duvick et al. (1998) were the first researchers to report the existence of microorganisms capable of metabolizing FB_1. The results of this study revealed that the first of at least two stages of FB_1 biodegradation is deesterification by a carboxylesterase, which results in hydrolyzed FB_1 (HFB_1), also known as aminopentol 1 (AP1). In this case, the bacterial strain was shown to metabolize $_{14}C$ from FB1 and release $_{14}CO2$.

In 2005, two promising genes for fumonisin degradation were identified in *Sphingopyxis* sp. MTA144. The genes revealed homologous sequences with B-type carboxylesterases and with aminotransferases, enzymes involved in the first and second steps of fumonisin degradation (the second step is the deamination of HFB_1, in the presence of pyruvate and pyrodoxal phosphate). The enzymatic activities for FB_1 hydrolyis and HFB_1 deamination of these enzymes were confirmed by liquid chromatography-mass spectrometry analysis (Heinl et al., 2010).

Recent research on these genes allowed the development of an enzyme-based food additive (FUMzyme®, Biomin, Tulln, Austria), composed mainly of the fumonisin esterase FumD (EC 3.1.1.87). This additive was produced from a genetically modified strain of *Komagataella pastoris*, which was evaluated for safety and efficiency in detoxifying FB_1 by the European Food Safety Authority (EFSA) and some research teams (Bampidis et al., 2020). In addition, other researchers identified a new fumonisin detoxification enzyme, FumDSB, from a *Sphingomonadales* bacterium expressed in *E. coli*. In this case, FB*1* is degraded by FumDSB to form HFB_1 by releasing two tricarboxylic acid groups (Li et al., 2021).

The enzyme's thermostability is essential for industrial applications because of special processes such as the pelletizing process. In the latter study, FumDSB remained at 76 and 58% relative activity at 40 and 50 °C for 10 min. Thus, compared with three different carboxylesterases already described, the authors conclude that FumDSB has adequate reaction conditions, excellent pH stability and thermostability, which enables the technological application of this enzyme as an ideal candidate in the food and animal feed industries (Li et al., 2021). This fact opens promising perspectives for application in beer processing.

In conclusion, it can be mentioned that with the reviewed articles it was performed network analysis. Specifically, the objective of this analysis was to verify the behavior of bibliometric networks for the selected articles, investigate possible hidden information and identify trends and relationships of the subject in question. The analysis is presented in Figure 2 and shows the network of co-citations of the co-authors according to the bibliographic portfolio.

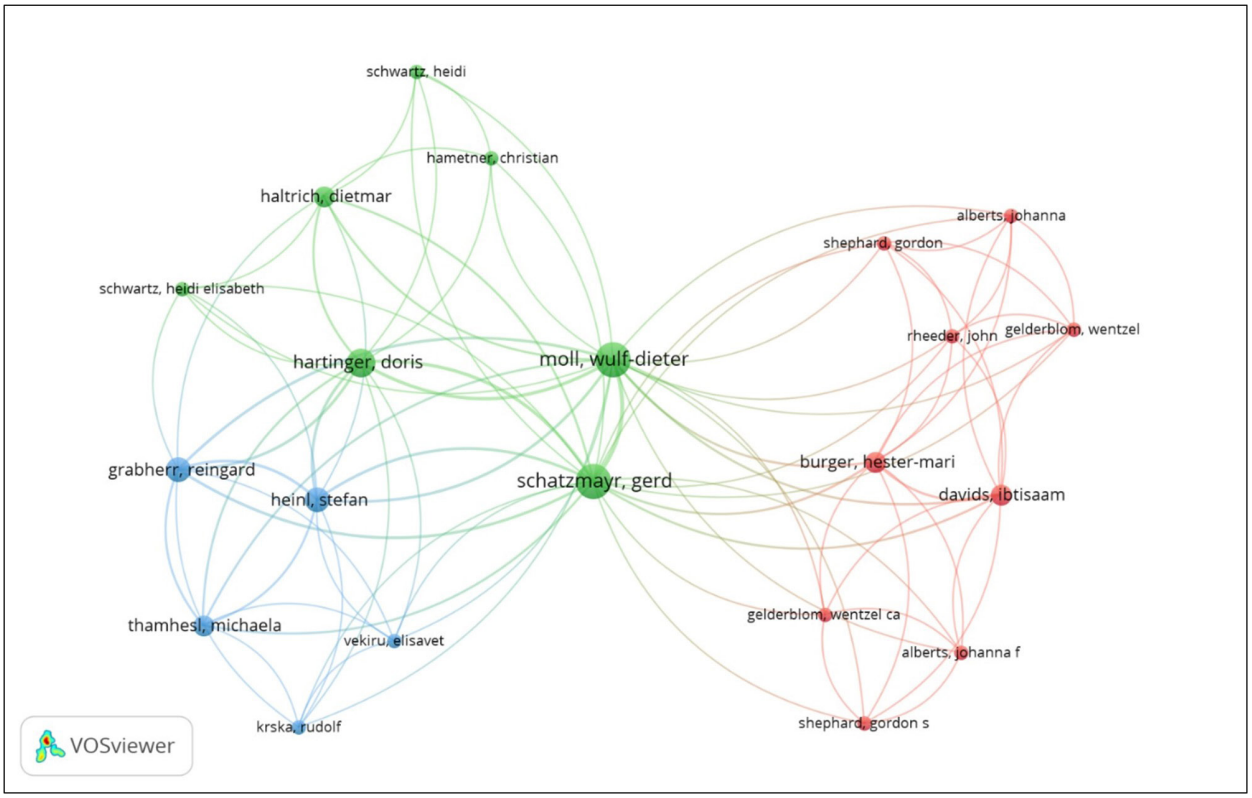


Figure 2. Co-authors network related to the biological control of fumonisins in beer production.

The co-authors network in the bibliometric analysis showed the formation of 3 groups (Figure 2).

The groups were formed specifically by the years of their publication: group 1 (red) brings together more recent works (published after 2017): group 2 (green) presents works published between 2013 and 2016; finally, group 3 (blue) contains works published before 2013. The authors with the most connections are Moll Wolf Dieter and Gerd Schatzmay. The first one relates to 20 works and with the 3 groups, while the Gerd Schatzmay study relates to 11 works and 2 groups (blue and green).

Conflict of interests

The authors declare that there are no known conflicts of interest associated with this publication.

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Supplementary Material

Supplementary material accompanies this paper.
MS 1 – Portfolio articles ranking on the occurrence of fumonisins using the InOrdinatio equation
MS 2 – Portfolio articles ranking of fumonisin processing using the InOrdinatio equation
MS 3 – Portfolio articles ranking on fumonisin control using the InOrdinatio equation
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