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Incidence of *Saccharomyces cerevisiae* var. *diastaticus* in the Beverage Industry: Cases of Contamination, 2008–2017

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ABSTRACT

Saccharomyces cerevisiae var. *diastaticus* is an obligatory spoilage microorganism in the beverage industry with high spoilage potential owing to its glucoamylase activity. *S. cerevisiae* var. *diastaticus* yeast strains can lead to an increase of the carbon dioxide concentration in beverages, with gushing and bottle bursting, caused by their superattenuating ability, as possible consequences. Therefore, the determination of beverage contamination by *S. cerevisiae* var. *diastaticus* is of significant interest. This article gives an overview of the incidence of *S. cerevisiae* var. *diastaticus* contamination in Europe over the last 9.5 years (2008–2017). The paper is based on analytical data of about 126 *S. cerevisiae* var. *diastaticus* instances in 52 companies (anonymous) in Europe recorded by the Research Center Weihenstephan for Brewing and Food Quality of the Technical University of Munich, presented according to their origin (beer, beer-mixed beverages, nonalcoholic beverages, etc.), country, year, and type of contamination. The accredited microbiological laboratory of the Research Center Weihenstephan investigates approximately 15,000 microbiological samples of the beverage industry worldwide per year. Real-time polymerase chain reaction analysis for *S. cerevisiae* var. *diastaticus* was conducted and evaluated. About six positive contaminations were detected every year, and 71% of them were caused by contamination events during the filling process of beverages. An increase in contamination incidents and confirmed positive findings can be observed over the last two years. Most problems with *S. cerevisiae* var. *diastaticus* contaminations occurred during the third quarter of the year. This analytical evaluation clearly shows the increase in contamination with *S. cerevisiae* var. *diastaticus* and the importance of detection in the breweries and the beverage industry in general.

Keywords: *Saccharomyces cerevisiae* var. *diastaticus*, Brewing yeasts, Spoilage yeast, Yeast characterization, Superattenuating

Introduction

The aim of every brewer is to manufacture a reproducible and flawless beer. Problems, particularly microbiological issues, can spoil the product. Beer spoilage microorganisms are therefore assessed in terms of their spoilage potential. The most hazardous and widespread of beer spoilage organisms belong to the class of so-called obligate beer spoilage organisms, which are still able to propagate in beer despite the low pH value, bitter substances in the hops, and very low oxygen concentrations. These organisms change the product both in terms of its physical-chemical and sensory properties as a result of metabolization. In addition to beer-spoilage bacteria such as *Lactobacillus brevis*, *Pectinatus*, and *Megasphaera*, so-called beer spoilage yeasts also play an often forgotten role (11).

Beer spoilage yeasts belong to both *Saccharomyces* and non-*Saccharomyces* types. *Saccharomyces* beer-spoiling yeasts are often regarded as being the most hazardous because they are difficult to differentiate from *Saccharomyces* brewing strains and directly compete with the culture strains. In particular, *Saccharomyces cerevisiae* var. *diastaticus* is considered to be an obligatory spoilage microorganism and spoilage yeast (e.g., wild yeast) in beer and beer-mixed beverages (2,7,12). Compared with *Saccharomyces* brewing culture yeasts, *S. cerevisiae* var *diastaticus* yeast strains are able to metabolize residual carbohydrates in naturally conditioned beers such as complex dextrins and starches. This physiological property is considered to be connected to *STA* genes encoding for the enzyme glucoamylase (1,12,14,18). These amylolytic strains of *Saccharomyces* are classified by classical taxonomic criteria to be a separate species from *S. cerevisiae.* However, genetic differences do not make such a clear separation, and genome sequence data unequivocally show that they are strains of the species *S. cerevisiae* (15). Despite multilocus and genome sequencing to solve taxonomic problems, many archaic and misleading synonyms are unfortunately still in use (8). Up to now, there is no correct taxonomic term for superattenuating yeasts of the genus *Saccharomyces*, but the term *S. cerevisiae* var. *diastaticus* is widely used throughout several publications (3,13,16,17). Contamination with *S. cerevisiae* var. *diastaticus* can cause changes in taste, sediment formation, turbidity, gushing, and swelling of the package. Additional fermentation in filled containers (such as bottles, cans, kegs, or disposable drums) can further cause a sharp increase in the carbon dioxide concentration in the product, and bottle bursting can be the consequence (5,21). In Germany, breweries and beverage manufacturers are legally obliged to report positive analysis results and potentially to remove sold products from the market in a public product recall. In cases of recurrent contamination and if entire batches are affected, the contamination source can usually be located and eliminated by microbiological monitoring. Affected batches are not placed on the market. Contamination in the bottling area is generally the greater evil. This type of contamination generally occurs as so-called scatter

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contamination and only affects single or several bottles. Such contamination can occur by aerosol infection owing to hygienic problems of the filler environment or by a so-called wash-out effect of biofilms in the pipework system of the filler. These kinds of trace contaminations are difficult to detect, and this is why finding evidence of *S. cerevisiae* var. *diastaticus* before placing the contaminated product on the market is so difficult. A microbiological contamination with *S. cerevisiae* var. *diastaticus* poses a significant threat to breweries and consumers alike. Product recalls owing to microbiological contaminations with *S. cerevisiae* var. *diastaticus* can cause economic losses and occasionally expose the consumer to risk of injury (4). The purpose of the work presented in this paper is to determine beverage contaminations by *S. cerevisiae* var. *diastaticus* and therefore to show the increase and the importance of detection in breweries and the beverage industry in general. Additional knowledge about the type of contamination and the time of the year *S. cerevisiae* var. *diastaticus* contaminations mainly occur can be helpful for brewers and beverage technologists to avoid microbiological problems. The paper further shows the increasing significance of *S. cerevisiae* var. *diastaticus* as a spoilage yeast.

Materials and Methods

Data Collection and Evaluation

The data collected are based on analysis data at the Technical University of Munich at the Research Center Weihenstephan for Brewing and Food Quality (BLQ). The accredited microbiological laboratory of the BLQ investigates approximately 15,000 microbiological samples of the beverage industry worldwide per year. Instances of *S. cerevisiae* var. *diastaticus* were collected from 2008 to June 2017 since the real-time polymerase chain reaction (PCR) for *S. cerevisiae* var. *diastaticus* was introduced. Instance was defined as being all investigated samples of the same customer/company when contamination with superattenuating yeast was suspected. Therefore, we defined a *S. cerevisiae* var. *diastaticus* instance as when we investigated a suspected *S. cerevisiae* var. *diastaticus* problem in a brewery on site, or when we received multiple samples from one specific brewery within a 3 month period. The result can be positive (positive finding) or negative (negative finding). Positive findings were evaluated as being only those analyses in which real-time PCR provided positive evidence of *S. cerevisiae* var. *diastaticus* (target DNA region *STA1* gene, method described in the next section). Further positive findings for microbiological organisms besides *S. cerevisiae* var. *diastaticus* are not shown and will not be considered in the article. The sample distribution and the number of samples sent to the BLQ depended on the customers/companies. The distribution had a shift depending on the number and structure of customers. Approximately 50% of the customers were German breweries and 35% were from other European countries. As a result, the number of positive findings was always related to the total number of samples sent. The terms primary and secondary contamination identify the type of contamination. Primary contaminations were defined as contamination of the wort, of the yeast (yeast crop and Kräusen addition), of the fermentation, bright beer, and storage tanks, and of the filtration systems used. Secondary contamination was defined as contaminations owing to hygienic problems of the filler environment and the filler hygiene itself. Such contaminations can be traced back to biofilms in the pipework system of the filler or can occur as an aerosolization of contaminating organisms owing to areas of the filler with spoilage organisms, which can become aerosolized by the vortex action of the filler, and dysfunctional drains near the filler that are not draining properly or not maintained with any regularity.

DNA Extraction and Real-Time PCR

DNA isolation and real-time PCR were performed according to the methods of Hutzler and Meier-Dörnberg (9,10,19,20). Specific real-time PCR system patterns can identify *S. cerevisiae* var. *diastaticus*. A specific primer and probe system is situated on the glucoamylase *STA1* gene (1). To amplify the specific rDNA region, the primers Sd-f (5´-TTCCAACTGCA CTAGTTCCTAGAGG-3´) and Sd-r (5´-GAGCTGAATGGAG TTGAAGATGG-3´) and the probe Sdia (5´-FAM-CCTCCTCT AGCAACATCACTTCCTCCG-BHQ1-3´) were used according to the method of Brandl (6). Other systems were also applied to prove the species identity *S. cerevisiae* and the absence of other *Saccharomyces* species. All real-time PCR reactions were performed from single colonies that were obtained as described below. Process and product samples that showed yeast contamination (turbidity, CO_2 development, off-flavor, microscopic picture) were plated on yeast mold (YM) agar, and single colonies were picked for real-time PCR identification. YM agar was produced according to the method of Hutzler (9) (3.0 g of broth malt extract, 3.0 g of yeast extract, 5.0 g

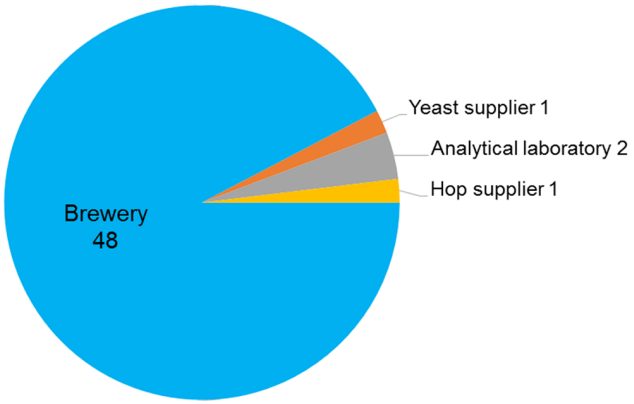

Figure 1. Total number of companies (n = 52) according to type of operation.

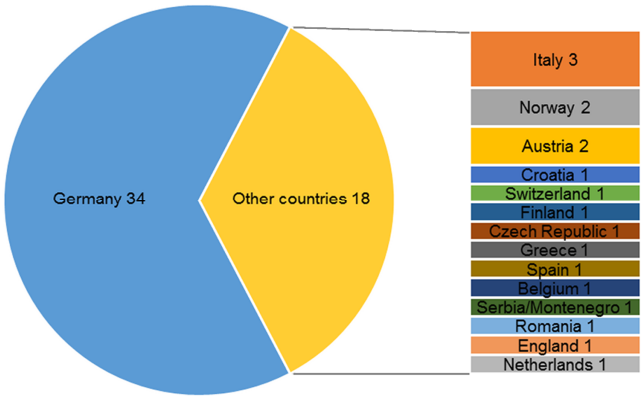

Figure 2. Total number of companies (n = 52) categorized according to operating site (countries) from 2008 to June 2017.

of peptone, 11.0 g of glucose monohydrate, 20.0 g of agar, and 1,000 mL of distilled water, incubated aerobic at 20°C for 5 days). In some cases a liquid preenrichment of yeast was necessary (suspicious samples without obvious spoilage appearance).

- For universal preenrichment, double-concentrated YM broth (without agar) was mixed 1:1 with the liquid sample and incubated at 28°C for 5 days. Sediment was plated on YM agar to obtain single colonies.
- For preenrichment in samples that contained bottom-fermenting brewer's yeast, *S. pastorianus*, double-concentrated YM broth was mixed 1:1 with the liquid sample and incubated at 37°C (selective conditions that suppress *S. pastorianus* growth) for 5 days. Sediment was plated and incubated on YM agar (see above).
- For preenrichment in samples that contained top-fermenting brewer's yeast, *S. cerevisiae*, double-concentrated YM broth containing 400 ppm of $CUSO_4$ was mixed 1:1 with the liquid sample and incubated at 28°C (selective condi-

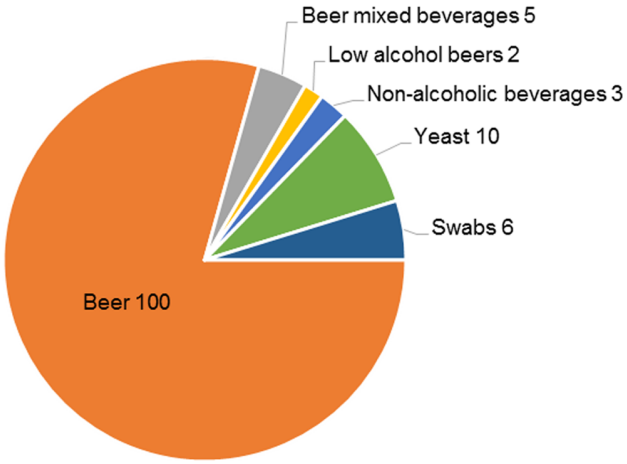

Figure 4. Total number of *S. cerevisiae* var. *diastaticus* instances (n = 126) categorized in a matrix for the period 2008 to June 2017.

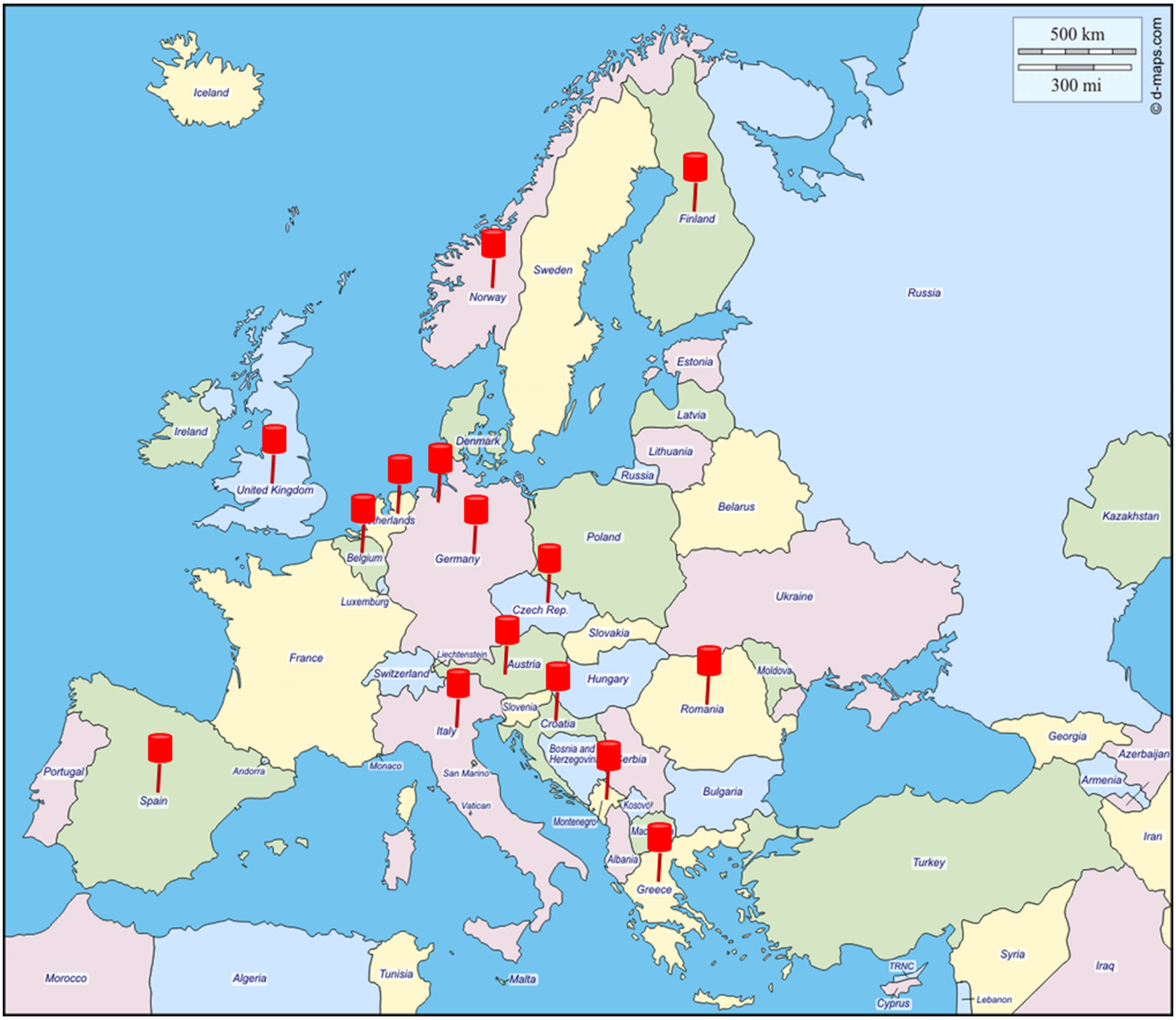

Figure 3. Overview of the investigations for *S. cerevisiae* var. *diastaticus* in Europe (source of the European map: http://d-maps.com/carte.php?num_car=13146&lang=en).

Table 1. Number of negative and positive findings of *S. cerevisiae* var. *diastaticus* per year from January 2008 to June 2017

Year	Negative finding	Positive finding	Total per year
2008	0	1	1
2009	0	1	1
2010	4	4	8
2011	11	4	15
2012	4	5	9
2013	7	4	11
2014	3	3	6
2015	10	17	27
2016	18	19	37
01–06/2017	7	4	11
Total 01/2008 to 06/2017	64	62	126
Mean/month for 114 months	0.56	0.54	1.11

Figure 5. Findings of *S. cerevisiae* var. *diastaticus* (n = 126) for the period 2008 to June 2017.

tions of 200 ppm $CUSO_4$ in final mixed liquid that suppress *S. cerevisiae* growth) for 5 days. Sediment was plated and incubated on YM agar to obtain single colonies (see above).

All real-time PCR results that were performed with the specific *STA1* gene real-time PCR system between 2008 and June 2017 are shown in our study. Species identity was confirmed by other real-time PCR systems as well. Negative results can occur when there are suspicious batches, single samples, or process steps that might be contaminated but *S. cerevisiae* var. *diastaticus* could not be proved in these samples (even with preenrichment steps).

S. cerevisiae var. *diastaticus* Instances from 2008 to June 2017

Total Number of Companies Categorized According to Type of Company and Operating Site (Country)

In the past 9.5 years (2008–2017), samples from a total of 52 different businesses were analyzed for contamination with *S. cerevisiae* var. *diastaticus* (positive and negative findings listed). With the exception of two analytical labs, a hop supplier, and a yeast supplier, these businesses were small-scale and large breweries (Fig. 1).

Of these 52 companies, 65% (i.e., 34 companies) were based in Germany, and the remaining 35% (18 companies) were spread over 14 other European countries. Three customers had their operating site in Italy, two in Austria, and two in Norway. The remaining 11 companies were spread over another 11 European countries. PCR analysis for *S. cerevisiae* var. *diastaticus* was performed for all 52 companies, and therefore the *S. cerevisiae* var. *diastaticus* instances are distributed across 15 countries in Europe (Figs. 2 and 3).

Incidence and Findings in the Beverage Industry

As Figure 4 shows, a total of 126 *S. cerevisiae* var. *diastaticus* instances were investigated within the last 9.5 years (2008

Figure 6. Number of negative and positive findings of *S. cerevisiae* var. *diastaticus* per year from 2008 to June 2017.

to June 2017). This corresponds to 13 instances a year on average or one instance per month (Table 1).

For the 52 companies in this period there was an average of nearly 2.5 incidents and one positive finding per company. It can therefore be assumed that, depending on the type (primary or secondary contamination), contamination with *S. cerevisiae* var. *diastaticus* is difficult to control using conventional brewery countermeasures (cleaning and disinfection) and/or the source of contamination could not be precisely located, which caused further incidents and therefore investigations for each company (Fig. 4).

As Figure 5 shows, 62 positive findings (just under half of the 126 investigated *S. cerevisiae* var. *diastaticus* instances) were established and confirmed.

Figures 6 and 7 show the general results and incidence of *S. cerevisiae* var. *diastaticus* during the period 2008 to June 2017. As shown in the figures, there was a significant increase in investigated instances of *S. cerevisiae* var. *diastaticus* starting in 2015 (27 of 126). In 2016, there were 37 investigated instances. At 19 positive findings, most of the *S. cerevisiae* var. *diastaticus* instances and confirmed contaminations over the last 9.5 years occurred in 2016.

Despite the lower number of investigations and confirmed findings so far in 2017 (first six months), a further increase in investigations is expected compared with 2016, because Figure 7 and Table 2 show that the majority of investigations took place in the late summer months of July to September and therefore in the third quarter of the year.

Type of Contamination in the Positive Findings

Table 3 categorizes the positive findings from 2008 to June 2017 according to their contamination type. In total, 18 of the 62 positive findings were attributed to the brewhouse, fermentation cellar, and storage cellar and were therefore classified as primary contamination.

Most of the confirmed contaminations, with a total of 71%, consisted of secondary contamination: 44 out of the 62 positive findings for *S. cerevisiae* var. *diastaticus* were found in the bottling hall when filling the product in bottles, cans, or kegs. The secondary contamination can be traced back to contaminants in the filler environment (confirmed by swabs) and/or biofilms in the pipework system of the filler. Such contamination can occur by aerosol infection owing to hygienic problems of the filler environment and/or by the so-called wash-out effect of the biofilm in the pipework system of the filler. Aerosol infections usually occur as scattered contamination in single packages and containers (e.g., bottles, cans, and kegs), whereas contaminations owing to biofilms mostly infect the first few packages and containers after starting the filling process.

Incidence and Findings in the Beverage Industry, According to the Matrix

As Figure 8 and Table 4 show, 93.5% of all the 62 proven *S. cerevisiae* var. *diastaticus* findings relate to direct contamination of the product. In total, 56 positive findings were evidenced in beer and two positive findings in beer-mixed beverages.

In addition to these 58 product contaminations (56 beer, two beer-mixed beverages), a further two positive findings were related to the yeast used (pitching yeast and Kräusen addition) and another two positive findings to swab samples taken in the bottling area. No positive result could be detected in the two analyzed low-alcohol beers or the three analyzed nonalcoholic beverages such as lemonade, cola, and so on.

Table 5 shows the number of positive and negative findings per matrix categorized according to the respective country

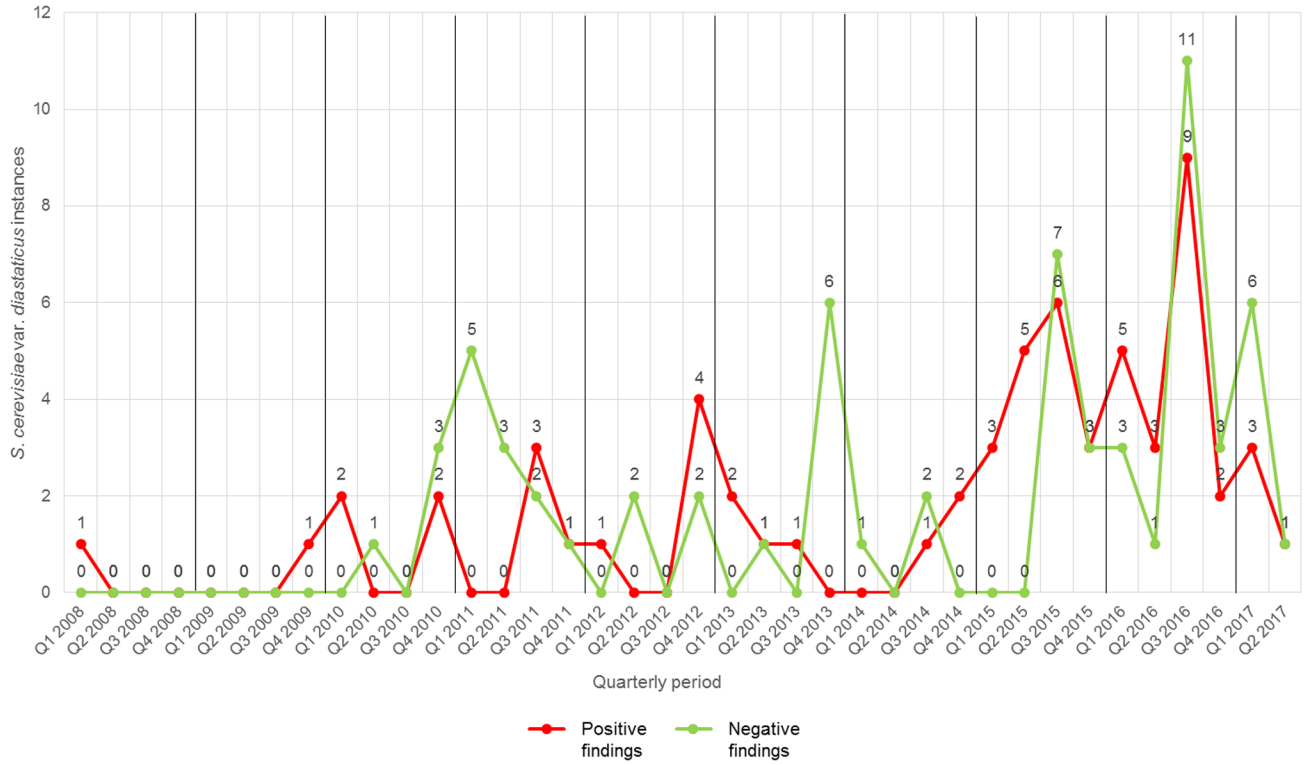

Figure 7. Number of negative and positive findings of *S. cerevisiae* var. *diastaticus* per quarter from 2008 to June 2017.

from which the samples came. As already illustrated in the section on data collection and evaluation, 34 of the 52 clients had their operating site in Germany. As evident from the table, the highest numbers of positive and negative cases were reported in Germany. A total of 60 cases were investigated, and 37 of these cases were analyzed directly in the beer product. Within the categories of beer-mixed beverages, low-alcohol beers, and nonalcoholic beverages, all analyzed cases were reported to be in Germany. With a total of 31 *S. cerevisiae* var. *diastaticus* cases, Norway had the second highest level of investigated beer samples and samples overall.

Positive Findings in Beer According to Country

All the confirmed contaminations (positive findings by real-time PCR) with *S. cerevisiae* var. *diastaticus* in beer are detailed and presented in Figure 9 and Table 6 according to country and year from 2008 to June 2017. The majority of beer contaminations occurred in 2015 and 2016, with 17 positive incidents in each case. In both years, most of the positive findings occurred in Norway.

In Norway, a total of 18 positive instances were recorded, 12 of which occurred in 2015 and six in the following year. Over the entire investigation period from 2008 to June 2017, Germany showed the most product contaminations, with a total of 23 positive findings spread over the years 2010 to June 2017. In England and Greece, beer samples were analyzed on the suspicion of contamination, but these were not confirmed as positive.

Number of Customers per Year Categorized According to Their Type of Business

From 2008 to June 2017, 126 instances of *S. cerevisiae* var. *diastaticus* were reported and then investigated. These cases were spread over a total of 52 different companies, which means that several companies reported multiple cases. Table 7 shows the total number of cases and customers per year categorized according to type of operation. The difference between the total number of 52 companies and the listed total number of 71 customers is owing to the year-dependent evaluation. The same company is evaluated as just one customer, if it has commissioned multiple analyses in the same year.

As already shown in the section on DNA extraction and real-time PCR, the highest number of cases was 37, reported and investigated in 2016. These cases were distributed across a total of 17 different small- and large-scale breweries, which

Table 2. Number of negative and positive findings of *S. cerevisiae* var. *diastaticus* per quarter from 2008 to June 2017

Quarter	Negative finding	Positive finding	Total	Total per year
01–03/2008	0	1	1	1
04–06/2008	0	0	0	
07–09/2008	0	0	0	
10–12/2008	0	0	0	
01–03/2009	0	0	0	1
04–06/2009	0	0	0	
07–09/2009	0	0	0	
10–12/2009	0	1	1	
01–03/2010	0	2	2	8
04–06/2010	1	0	1	
07–09/2010	0	0	0	
10–12/2010	3	2	5	
01–03/2011	5	0	5	15
04–06/2011	3	0	3	
07–09/2011	2	3	5	
10–12/2011	1	1	2	
01–03/2012	0	1	1	9
04–06/2012	2	0	2	
07–09/2012	0	0	0	
10–12/2012	2	4	6	
01–03/2013	0	2	2	11
04–06/2013	1	1	2	
07–09/2013	0	1	1	
10–12/2013	6	0	6	
01–03/2014	1	0	1	6
04–06/2014	0	0	0	
07–09/2014	2	1	3	
10–12/2014	0	2	2	
01–03/2015	0	3	3	27
04–06/2015	0	5	5	
07–09/2015	7	6	13	
10–12/2015	3	3	6	
01–03/2016	3	5	8	37
04–06/2016	1	3	4	
07–09/2016	11	9	20	
10–12/2016	3	2	5	
01–03/2017	6	3	9	11
04–06/2017	1	1	2	
Total	64	62	126	126

Table 3. Number of findings of *S. cerevisiae* var. *diastaticus* and type of contamination per year from 2008 to June 2017

	Finding		Type of contamination and number of positive findings[a]	
Year	Negative	Positive	Primary	Secondary
2008	0	1	0	1
2009	0	1	0	1
2010	4	4	2	2
2011	11	4	1	3
2012	4	5	1	4
2013	7	4	1	3
2014	3	3	0	3
2015	10	17	5	12
2016	18	19	7	12
01–06/2017	7	4	1	3
Total	64	62	18	44
	126		62	

[a] Primary contamination is in the brewhouse, fermentation cellar, and storage cellar, and secondary contamination is in the bottling hall.

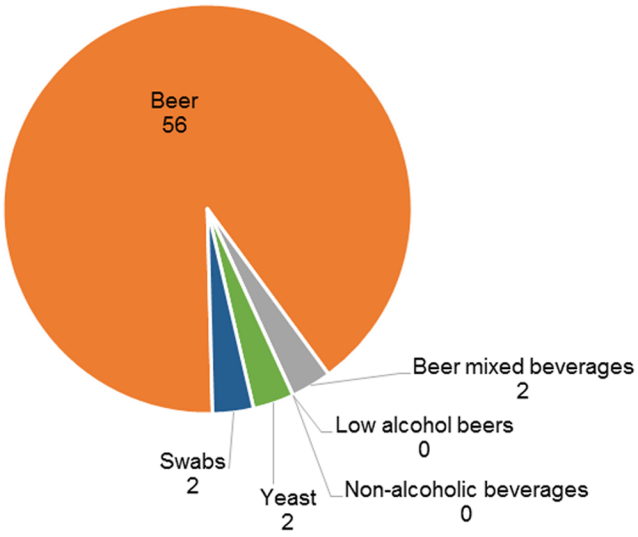

Figure 8. Total number of *S. cerevisiae* var. *diastaticus* positive findings ($n = 62$) characterized by matrix for the period 2008 to June 2017.

Table 4. Number of negative and positive findings of *S. cerevisiae* var. *diastaticus* per matrix per year from January 2008 to June 2017

	Beer		Beer-mixed beverage		Low-alcohol		Nonalcoholic beverage		Yeast		Swab		
Year	**–**	**+**	**–**	**+**	**–**	**+**	**–**	**+**	**–**	**+**	**–**	**+**	**Total**
2008	0	1	0	0	0	0	0	0	0	0	0	0	1
2009	0	0	0	1	0	0	0	0	0	0	0	0	1
2010	3	4	0	0	0	0	0	0	1	0	0	0	4
2011	5	3	2	0	0	0	0	0	4	0	0	1	4
2012	2	5	1	0	0	0	0	0	0	0	1	0	5
2013	6	2	0	1	0	0	0	0	1	1	0	0	4
2014	1	3	0	0	0	0	1	0	1	0	0	0	3
2015	10	17	0	0	0	0	0	0	0	0	0	0	17
2016	11	17	0	0	2	0	2	0	0	1	3	1	19
01–06/2017	6	4	0	0	0	0	0	0	1	0	0	0	4
Total	44	56	3	2	2	0	3	0	8	2	4	2	126
Total	100		5		2		3		10		6		126

Table 5. Number of positive findings of *S. cerevisiae* var. *diastaticus* per country categorized in the matrix from January 2008 to June 2017

	Beer		Beer-mixed beverage		Low-alcohol		Nonalcoholic beverage		Yeast		Swab		
Country	**–**	**+**	**–**	**+**	**–**	**+**	**–**	**+**	**–**	**+**	**–**	**+**	**Total**
Germany	18	19	3	2	2	0	3	0	7	1	3	2	60
Italy	2	4	0	0	0	0	0	0	0	0	0	0	6
Norway	13	18	0	0	0	0	0	0	0	0	0	0	31
Austria	3	2	0	0	0	0	0	0	0	0	0	0	5
Croatia	0	1	0	0	0	0	0	0	0	0	0	0	1
Switzerland	0	1	0	0	0	0	0	0	1	0	0	0	2
Finland	0	1	0	0	0	0	0	0	0	0	0	0	1
Czech Republic	0	1	0	0	0	0	0	0	0	0	0	0	1
Greece	1	0	0	0	0	0	0	0	0	0	0	0	1
Spain	0	1	0	0	0	0	0	0	0	0	0	0	1
Belgium	0	1	0	0	0	0	0	0	0	0	0	0	1
Serbia/Montenegro	0	1	0	0	0	0	0	0	0	0	0	0	1
Romania	1	2	0	0	0	0	0	0	0	0	0	0	3
England	1	0	0	0	0	0	0	0	0	0	0	0	1
Netherlands	5	4	0	0	0	0	0	0	0	1	1	0	11
Total	44	56	3	2	2	0	3	0	8	2	4	2	126
Total	100		5		2		3		10		6		126

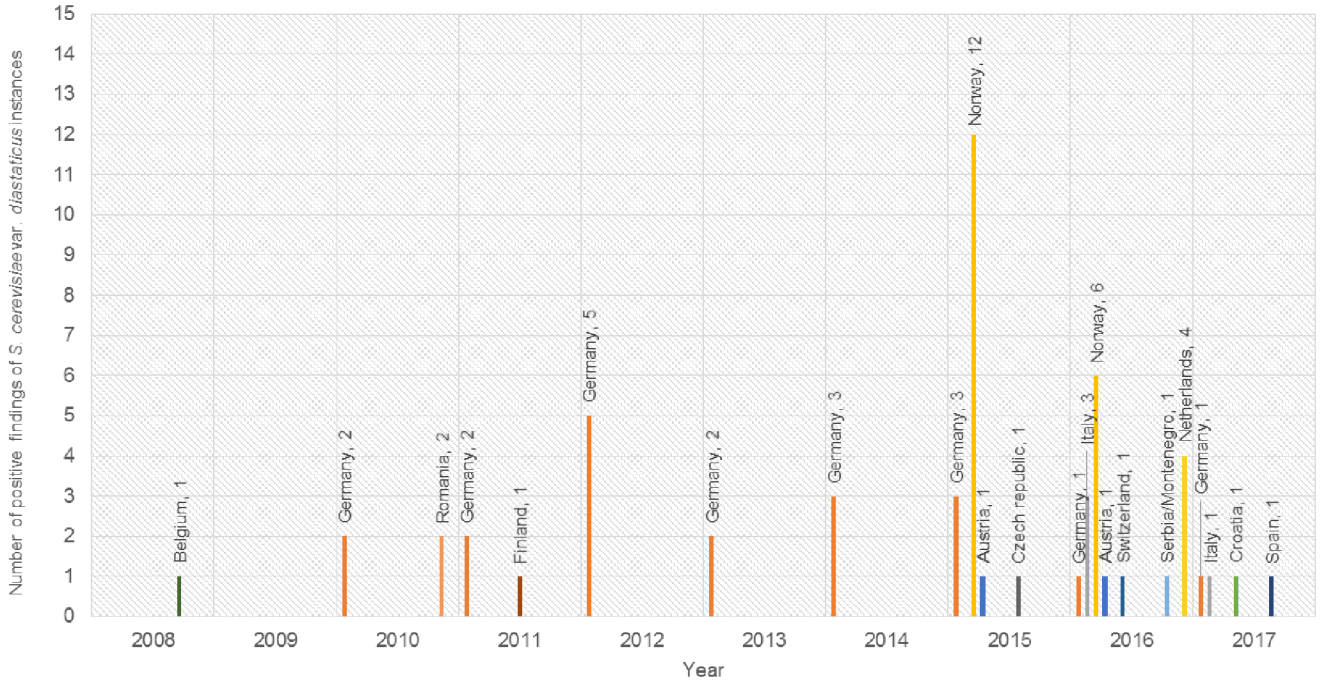

Figure 9. Number of positive findings of *S. cerevisiae* var. *diastaticus* in beer per year and country from 2008 to June 2017.

Table 6. Number of positive findings of *S. cerevisiae* var. *diastaticus* in beer per year and country from January 2008 to June 2017

Year/country	2008	2009	2010	2011	2012	2013	2014	2015	2016	01–06/2017	Total
Germany	0	0	2	2	5	2	3	3	1	1	19
Italy	0	0	0	0	0	0	0	0	3	1	4
Norway	0	0	0	0	0	0	0	12	6	0	18
Austria	0	0	0	0	0	0	0	1	1	0	2
Croatia	0	0	0	0	0	0	0	0	0	1	1
Switzerland	0	0	0	0	0	0	0	0	1	0	1
Finland	0	0	0	1	0	0	0	0	0	0	1
Czech Republic	0	0	0	0	0	0	0	1	0	0	1
Greece	0	0	0	0	0	0	0	0	0	0	0
Spain	0	0	0	0	0	0	0	0	0	1	1
Belgium	1	0	0	0	0	0	0	0	0	0	1
Serbia/Montenegro	0	0	0	0	0	0	0	0	1	0	1
Romania	0	0	2	0	0	0	0	0	0	0	2
England	0	0	0	0	0	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0	0	0	4	0	4
Total	1	0	4	3	5	2	3	17	17	4	56

Table 7. Number of customers per year categorized according to type of company from January 2008 to June 2017

Year	Number of instances	Number of customers	Brewery	Yeast supplier	Analytical lab	Hop supplier
2008	1	1	1	0	0	0
2009	1	1	1	0	0	0
2010	8	3	2	0	1	0
2011	15	11	10	0	1	0
2012	9	6	6	0	0	0
2013	11	9	7	1	1	0
2014	6	6	6	0	0	0
2015	27	8	7	0	1	0
2016	37	17	17	0	0	0
01–06/2017	11	9	8	0	0	1
Total	126	71	65	1	4	1

also represent the main customer base, overall and per year. In 2013, a yeast supplier and an analytical laboratory, in addition to breweries, also sent samples to investigate contamination with *S. cerevisiae* var. *diastaticus*. In the current year, 2017, the customer base also included a hop supplier.

Summary

Overall, 126 cases from a total of 52 companies from 15 countries in Europe were investigated for contamination with *S. cerevisiae* var. *diastaticus* over the past 9.5 years (January 2008 to June 2017), and 62 of these cases were confirmed with a positive result for strains of this yeast. With 71% in total, most confirmed cases (real-time PCR *STA1* gene positive) occurred as secondary contamination in the bottling area (bottling hall) and were attributed to contaminants in the filler environment (confirmed by swabs) and/or to biofilms in the pipework system of the filler. Just 29% of cases related to primary contamination in the brewhouse, fermentation cellar, and storage cellar. Beer, beer-mixed beverages, nonalcoholic beverages, low-alcohol beers, yeast samples, and swabs from breweries, yeast suppliers, hop suppliers, and analytical labs were investigated. Starting in 2015, an increase was recorded in contaminations and confirmed positive results, showing the most cases in the third quarter of each year.

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