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Escherichia coli in flour – sources, risks and prevention

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Flour is a natural product and a valuable foodstuff. However, Shigatoxin-producing *Escherichia coli* (STEC) were detected in multiple flour samples (wheat, spelt and rye) during routine food monitoring in Germany in 2018.

Escherichia (*E.*) *coli* are bacteria that occur naturally in the intestines of animals and humans and the detection of *E. coli* in food is a strong indicator of a faecal contamination. Bacteria from the faeces or stool can be shed into the environment and subsequently contaminate various animal- and plant-based foods. Direct transmission between animals and humans and from humans to humans are also possible. Certain toxin producing variants of *E. coli* can cause serious diseases in animals and humans.

E. coli variants that can form Shiga toxins are of particular importance for humans. These are abbreviated as STEC. STEC, which cause diseases in humans, are referred to as enterohemorrhagic *E. coli* (EHEC).

The symptoms of an infection with STEC are initially gastrointestinal. The possible severity of the disease ranges from watery to bloody diarrhea. In adults, the course of the disease can also proceed without symptoms. A particularly severe complication is the hemolytic-uremic syndrome (HUS). HUS is a disease that manifests in acute kidney failure, blood coagulation disorders and destruction of the red blood cells and can lead to death in individual cases. This form of the disease affects particularly sensitive groups of people, such as young children.

The BfR therefore advises consumers who wish to protect themselves and their families from food infections to follow the hereafter listed recommendations when handling flour, in addition to standard kitchen hygiene rules:

- Before preparing food and after contact with flour, wash hands thoroughly with soap and water and dry carefully.
- Where possible, avoid contact between flour and food for direct consumption, also use different boards, plates, bowls and stirrers or wash them after contact with flour.
- Clean surfaces and objects thoroughly with detergent and warm water after contact with flour, and dry them.
- Do not eat unbaked cake and cookie dough.

EHEC/STEC are killed by cooking, roasting and stewing. In general, when preparing food in private households by boiling or frying, a temperature of at least 70°C in the core of the food held for at least two minutes is sufficient. It should be noted that these values do not apply to the application of dry heat (without water) and are also insufficient for heating dough. In the dry flour product (approx. 13 % water content) STEC are not killed at 70 °C. These bacteria are also relatively insensitive to acids, low temperatures or dehydration. Therefore, even in the freezer, STEC bacteria cannot be reliably killed.
If flour is mixed with egg, milk or water to form a dough, STEC bacteria can be killed at core temperatures of 70 °C for at least two minutes. Higher core temperatures can reduce the necessary heating time.

However, there is still a great need for research, so that a final health risk assessment is not yet possible. The BfR is planning a meeting with selected experts to discuss the open scientific questions on STEC in flour.

BfR risk profile: [Escherichia coli in flour – sources, risks and prevention] (Opinion No. 004/2020)					
A Affected persons	General population young children people with weakened immune systems [1]				
B Probability of impairment to health due to EHEC/STEC in flour	Practically impossible	Unlikely	**Possible [2]**	Probable	Certain
C Severity of impairment to health due to EHEC/STEC in flour	**No impairment**	**Slight impairment [reversible/irreversible]**	**Moderate impairment [reversible/irreversible]**	**Severe impairment [irreversible] [3]**	
D Validity of available data	High: The most important data is available and there are no contradictions	Medium: Some important data is missing or contradictory	**Low: Much important data is missing or contradictory**		
E Controllability by consumers	Controls not needed	**Controllable through precautionary measures**	**Controllable through avoidance**	Not controllable	

Fields marked in dark blue indicate the properties of the risk assessed in this opinion (for more details, see the text of Opinion No. [004./2020] of the BfR dated [20 January 2020]).

Row A – Affected persons:

[1] – Mainly toddlers and young children up to the age of 5, pregnant women and people with weakened immune systems.

Row B – Probability of an impairment to health

[2] – The probability of an impairment to health depends on whether and in what quantities the flour consumed contains EHEC/STEC. The treatment of the food containing flour (raw or baked) also has an influence. An impairment to health is possible with the consumption of dough (with raw flour). Individual factors for the consumer, such as the state of their immune system, also have an influence.

Row C – Severity of impairment to health

[3] – The severity of the disease depends on the type and quantity of EHEC/STEC ingested. Asymptomatic courses, mild disease courses with gastrointestinal tract symptoms, bloody diarrhoea and haemolytic-uraemic syndrome with kidney failure, up to and including fatalities, are all possible.

Row E – Controllability by consumers

[4] – The information given in the row 'Controllability by consumers' should not be regarded as a recommendation from the BfR; it has a purely descriptive character. Precautionary measures recommended by the BfR can be found in the grey box on the first page of this opinion, and in the section: Further information on the BfR website on the topic at the end of this opinion

1 Subject of the assessment

The Federal Ministry of Food and Agriculture (BMEL) has asked the German Federal Institute for Risk Assessment (BfR) to provide a statement on Shiga toxin-producing *Escherichia coli* (STEC) in flour. The request arose in the context of the nationwide monitoring plan of 2018, where flour samples from mills were investigated for the occurrence of STEC. In wheat, spelt

and rye flour samples, STEC was found quite frequently. STEC isolates submitted to the BfR for serotyping revealed in some cases serotypes seen earlier associated with human diseases.

The problem in question is: which risks are associated with the handling and the use of STEC-positive flours as well as the consumption of food manufactured from STEC-positive flours.

The focus of this health assessment on flour and doughs produced therefrom is on wheat flour, due to the fact that most of the data available are specific for this matrix. According to consumption data, among the different flour types the population has the highest exposure to wheat flour. Fine pastries, pasta (dry or fresh) and frozen goods with flour as a separating agent were not included in the current assessment.

2 Results

Analysis of publications and data from the official food control authorities in Germany has shown that STEC is detectable in a significant proportion (10 to 30 %) of flour samples.
A wide range of STEC serotypes was detected in flours, including human pathogenic types with different combinations of pathogenicity factors. STEC that cause diseases in humans are known as enterohemorrhagic *E. coli* (EHEC). EHEC infections can cause severe gastrointestinal disease in all population groups (but especially in young children) and the hemolytic uremic syndrome (HUS), the latter primarily seen in young children. In individual cases, both diseases can lead to death. The HUS bears additionally the risk of a lifelong requirement for dialysis treatment.
A link between disease outbreaks and STEC contamination in flour was established in the USA and Canada. Although thus far in Germany no such direct link has been shown, a close genetic match between an STEC isolate in flour and a human isolate (EHEC O157:H7) has been found. The BfR therefore concludes that the occurrence of highly pathogenic EHEC variants in flour is also probable in Germany. It is likely that such direct links have simply not yet been discovered.
The consumption of raw cookie dough plays a repeated role in infections with connection to STEC in flour. Although other routes of infection may also be considered (lack of kitchen hygiene, use of flour as a separating agent), the risk of infection from the consumption of raw cookie dough as a main source of infection is plausible. Raw cookie dough is seen as a trend food and is commercially offered and advertised for raw consumption in Germany. In Germany, commercially marketed cookie dough/cookie dough mixes for raw consumption are produced with pasteurized flour. However, little is known about the exact parameters and technology of the heat treatment and it is unclear whether proof of the effectiveness of the heat treatment can always be provided. For the effective elimination of bacteria, dry heat treatment must be more intensive than pasteurization of a liquid product.
The manufacturers state that there is no risk of infection for consumers posed by ready to use dough made from heat-treated/pasteurised flour. As the parameters and technology of the heat treatment are not known, the BfR cannot assess whether this assessment is correct.

Several stages of flour production are possible sources of STEC contamination. Contamination with STEC probably originates from an entry into the food chain in the field. Contamination of the primary product, e.g. by wild ruminants, plays a role here. Other possible sources of contamination include organic fertilization and irrigation. The mills play an important role in particular in the distribution of bacteria within a product batch and the spreading of the STEC from batch to batch. Therefore, mill hygiene is a promising starting point for minimizing the risk of STEC transmission through flour.
The microbiological status of ready-made doughs is unknown and is subject to a complex mixture of influencing factors such as bacterial entry through raw materials, e.g. flour, as well as specific bacterial reduction measures by the manufacturers. The BfR therefore suggests including the product group "ready-made doughs" in the national monitoring programs (MNKP, BüP) and/or zoonosis monitoring.

The BfR recommends the following risk mitigation measures to manufacturers of flours, baking mixtures and ready-made doughs:

1. Evaluation of the effectiveness of cleaning and disinfection plans (where applicable) on a regular basis via suitable self-monitoring programs. The programs should include tests of samples for the presence of STEC. An action plan must be ready for implementation if STEC is detected within the internal control program.
2. Heat treatment of ready-made dough for retail sale or production of ready-made dough from heat-treated flour (effectiveness of the heat treatment steps used should be demonstrated, including the reliable killing of STEC).
3. Ready-made doughs must not contain pathogenic bacteria.
4. Avoid and thoroughly remove flour dust wherever possible.
5. Avoid dusting of baked goods with flour after baking.

Further risk mitigation measures, such as conditioning the grain with hot steam, treating the grain with ozone or UV radiation or irradiating ready-made doughs, still require technological development and research with regard to effectiveness and influence on nutritional aspects and consumer acceptance:
The BfR recommends consumers protect themselves against food-borne infections by

1. Always storing and processing flour, baking mixes and raw dough separately from other foodstuffs.
2. Processing doughs as quickly as possible or storing them in the refrigerator.
3. Consuming doughs and baked goods only after thorough heating.
4. Cleaning kitchen utensils and surfaces thoroughly after contact with flour, baking mixtures or raw dough.
5. Washing and drying hands thoroughly after processing flour, baking mixes or raw dough.

To make the German public aware of the possible presence of pathogens in flour, existing consumer guidance should be amended accordingly. Facilities with a multiplier function and

particularly vulnerable groups of people (community facilities such as schools and day-care centres) should also receive appropriate informational material.

There is a need for research on the following questions:

- Pathways of pathogenic microorganisms into cereals and flour and
  - its dependence on the grain used (wheat, rye, spelt etc.)
  - its dependence on the primary production (cultivation [organic, conventional], cultivation area, irrigation, plot size, associated animal husbandry, occurrence of game etc.)
  - consideration of further contamination routes (water, personnel, insects, birds, rodents etc.)
- Prevalences of STEC in flours and flour-containing products depending on the type of flour and origin, production areas and transport routes
- Effectiveness of risk mitigation measures in the mill (prevalence in production environment, efficiency of cleaning and, if necessary, disinfection measures)
- Behaviour of pathogenic microorganisms and the microbiome in different types of dough (yeast dough, wafer dough, bread roll dough, ...) as a function of temperature and time
- Optimisation and standardisation of methods for isolating STEC from flour and flour products (standardisation of detection is particularly important for comparing prevalences)
- Importance of dormant cells for diagnostics, research on reactivation and/or detection of these cell stages
- Effectiveness of the applied technologies of heat treatment of flour for killing STEC
- Determination of prevalences of STEC in flour, taking different methods, contamination or technology-related germ reduction into account
- Investigation of alternative measures for inactivating STEC in flour, e.g. conditioning of grain with hot steam, treatment of grain with ozone or UV radiation and irradiation of ready-made doughs, with regard to effectiveness and influence on nutritional aspects as well as consumer acceptance

3 Rationale

3.1 Risk assessment

3.1.1 Shiga toxin-producing *Escherichia coli*

Escherichia (*E.*) *coli* is a Gram-negative, non-spore forming, usually motile rod shaped bacterium that belongs to the bacterial family of *Enterobacteriaceae*. It occurs naturally in the intestines of animals and humans and is therefore considered the most important indicator for faecal contamination. The detection of *E. coli* in food indicates inadequate processing, operational or distribution hygiene. Certain strains of *E. coli* can cause serious diseases in animals and humans. Of particular importance for humans are *E. coli* strains that can form Shiga toxins (Stx) (synonym: Verotoxins). Shiga toxin-producing *E. coli* (STEC) usually occur in the intestines of

ruminants such as cattle, sheep and goats, as well as in wild ruminants. Animals that excrete STEC usually do not show symptoms of disease. The bacteria are shed with the faeces into the environment to contaminate foodstuffs of animal or plant origin. Direct transmission between animals and humans and from human to human is also possible. STEC that cause diseases in humans are called enterohemorrhagic *E. coli* (EHEC).
Within the framework of official monitoring, STEC are most frequently detected in meat from wild ruminants (Hartung, Tenhagen et al. 2016). However, they have also been detected in plant-based foods and in foods with a low a_w-value, such as flour or hazelnuts. STEC can grow within the temperature range from 8-45 °C and require a_w-values of >0.95 for replication (Beuchat, Komitopoulou et al. 2013). They are resistant to drying and freezing, so they can survive in the environment (soil, water, faeces) for weeks or months. Experiments on STEC survival in flour show that all serotypes tested remained viable at room temperature (23 °C) for at least nine months. Viable *E. coli* O157:H7 were still detectable after one year (Forghani, den Bakker et al. 2018).
At temperatures above 60 °C, STEC begin to die. For *E. coli* O157:H7, D-values[1] are known for foods such as meat and milk. Similar to other types of *E. coli*, these are in the temperature range of 57 - 64 °C at times between 270 and 9.6 seconds. However, the fat content and drying of food can increase the D-value. In the laboratory, enhanced survival of STEC after drying (on paper) has been shown, with the bacteria surviving at 70 °C for 5 hours (Hiramatsu, Matsumoto et al. 2005). This effect likely also applies to survival in flour.
In decontamination experiments with *E. coli* O157:H7, contaminated food has been treated with 0.5, 1.0 and 1.5 % organic acids. The treatments have proven to be ineffective and therefore emphasize the acid tolerance of this pathogen (Brackett, Hao et al. 1994). STEC can also tolerate salt (Dupree, Price et al. 2019). The concentration of STEC on foods can be reduced with sulphur dioxide (SO2), which is permitted as a preservative and antioxidant for various foods (E220). Examples of the use of SO_2 are dried fruits, but also dried potato products or dried or frozen white vegetables. The permitted maximum levels are product-specific. In various sour apple juice products, for example, a reduction of *E. coli* O157:H7 of up to 5 log units can be achieved with the application of 50 ppm SO_2 (Basaran-Akgul, Churey et al. 2009).

3.1.2 Hazard potential from EHEC infections

EHEC can cause mild to severe diarrhea in humans. Small children are especially at risk of developing hemolytic-uremic syndrome (HUS) as a result of an infection. HUS is a disease that manifests itself in acute kidney failure and results for a significant proportion of patients in dialysis dependency. In individual cases (approx. 5 out of 1000 cases) an EHEC infection can lead to death. The following information on infectious dose, incubation time and excretion duration mainly refers to findings on EHEC of the serotype O157:H7. With bacterial counts of less than 100 colony forming units (CFU), the infectious dose is very low (Paton, Ratcliff et al. 1996, Tilden, Young et al. 1996, Teunis, Takumi et al. 2004). The incubation period is about 2-10 days (on average 3-4 days) (RKI 2011). Patients remain infectious as long as EHEC bacteria are detected in the stool. Data on the duration of bacterial shedding vary considerably,

[1] The D-value is the time required at a given temperature to reduce a population of microorganisms to 10 %.
a_w-value: activity of water; measure of the availability of water in food and/or dishes. The higher the a_w-value, the more water is available for the growth/metabolism of bacteria

from a few days to several weeks or months (Matussek, Einemo et al. 2016, Bai, Mernelius et al. 2018). In a Swedish study, a mean shedding duration of 17 days was determined (Bai, Mernelius et al. 2018).
In Germany, a total of 2020 reports on EHEC diseases and 95 HUS cases were submitted to the RKI in 2017. As in previous years, children under 5 years of age were particularly often affected (29 % of EHEC cases and 48 % of HUS cases). EHEC diseases are more common in female than in male persons. 15 % of EHEC cases reported for 2017 with serogroup information were caused by O91 strains, 13 % by O103 strains and 11 % by O157 strains. In 2017, eight confirmed deaths related to HUS diseases (approx. 8.4 per 100 cases) and two deaths (approx. 1 per 1000 cases) due to EHEC-related diarrhea were reported to the RKI (RKI 2019).

3.1.3 Exposure

3.1.3.1 Sources of STEC contamination in flour

STEC can be introduced into flour at several points in the food chain, for example during wheat cultivation, harvest, storage, and during processing of the grain. The processing steps include cleaning, tempering, grinding and packaging. The bacteria on the grain (wheat) are often seen as the main source of contamination (Sperber and Group 2007, Sabillón and Bianchini 2016). As additional possible origins, the fertilization of wheat fields with manure from STEC-colonized cattle and the input via faeces from wild ruminants have been discussed (Laidler, Tourdjman et al. 2013, Persad and LeJeune 2014). Among farm animals, cattle are the main source of Shiga toxin-producing *E. coli* (EFSA 2008). Human pathogens can be introduced into the soil through the application of organic fertilizers from animal husbandry (Olaimat and Holley 2012) and viable STEC have been re-isolated from artificially contaminated soils after more than 100 days (Ma, Ibekwe et al. 2011). Irrigation water may also be contaminated with STEC (Islam, Doyle et al. 2004). In a study on the contamination of wheat seeds, it was shown that *E. coli* O157:H7 could be detected in the seedling in two of 96 cases after contamination of the seed and subsequent germination on sterile soil (Martinez, Stratton et al. 2015). In another experiment, sterile seeds were placed in contaminated soil. In five of 50 seedlings, internalised *E. coli* O157:H7 were found. Experiments on the contamination of wheat ears via spray irrigation with contaminated water showed a substantial increase in the *E. coli* population on the wheat ears after 24 hours and a survival time of up to 15 days (endpoint of experiment). In this study, irrigation of wheat ears was identified as the most likely source of contamination under natural conditions due to the high survival rates of *E. coli* O157:H7 on wheat ears (Martinez, Stratton et al. 2015).
The entry of STEC into the mill is probably via dust-encrusted cereal grains or deposits in the transport/storage bins (Gilbert 2010).

3.1.3.2 Processing in the mill and effects on bacterial counts

After the grain has been stored, it undergoes further processing in the mill, including cleaning, conditioning (tempering) and actual grinding. During these steps, contamination or recontamination may spread within the process itself.

In a study from the USA, total aerobic bacterial counts of 0.9 to 8.4 log CFU per g were found in durum wheat grain samples, depending on the region and preceding rain (Manthey, Wolf-Hall et al. 2004). Others describe total aerobic counts in the range of 2 - 4.9 log CFU per g (Rogers and Hesseltine 1978). The initial (rough) cleaning of the grains by sieving did not significantly reduce the total aerobic bacterial count. However, a reduction of the total aerobic bacterial count by one power of ten was achieved by the use of brushes. Based on the samples with high bacterial counts it could be shown that the cleaning of contaminated grains resulted in an average reduction of one power of ten, though in exceptional cases an increase in the total aerobic bacterial count was observed. The microbial contamination was detected here on the husk of the grain, which was removed in the process (Manthey, Wolf-Hall et al. 2004).
During the conditioning (tempering) of the wheat grains, water is added to increase the water content of the grain to approx. 15 %. Depending on the type of wheat, conditioning takes six to 48 hours at 23-24°C. This improves the further processing properties. To achieve evenly wetted grains, spray nozzles are used and the material to be ground is mixed thoroughly. This also leads to cross-contamination and distribution of microorganisms within the material to be ground. According to the German Milling Association, the water used for conditioning must be of drinking water quality. The hygiene guidelines for grain mills issued by the German Milling Association also recommend regular checks of the process step tempering and, if necessary, cleaning to prevent the undesirable multiplication of germs (Verband Deutscher Mühlen [Association of German Mills] e.V. 2016).
No significant increase in the total aerobic bacterial count was observed during conditioning in an experimental system. However, after conditioning, bacteria were detected that had not been detectable before and a significant increase in *E. coli* was shown. This suggests a selective enrichment of bacteria whose total aerobic bacterial counts before conditioning were below the detection limit or were inhomogeneously distributed. It is also possible that the bacteria were revived from a non-culturable but nevertheless infectious state (Berghofer, Hocking et al. 2003, Scherber, Schottel et al. 2009). After the process, grain deposits in tempering cells, elevators (screw conveyers for transport) and storage bins were noted (Berghofer, Hocking et al. 2003).
Grinding reduces the total bacterial count because the husk of the grain is removed. However, during the separation of the individual parts of the grain, mixing occurs. Subsequently, approx. 90 % of the total aerobic bacterial count is found in the core husk (bran), which is then separated. Further studies show that by grinding the peeled grain a further reduction of the total aerobic bacterial count of about one log unit can be achieved (Rogers and Hesseltine 1978, Manthey, Wolf-Hall et al. 2004).
On the other hand, heat is generated during grinding and, as a consequence, condensation water is produced. This can promote the growth of microorganisms (Posner 2005).
Further processing steps, such as the drying of spaghetti, again showed a bacterial reduction of 2-3 log units compared to the initial product, durum wheat semolina (Manthey, Wolf-Hall et al. 2004).
High grain quality (weight, grain integrity) in durum wheat was associated with low total aerobic bacterial counts (Manthey, Wolf-Hall et al. 2004). In summary, the introduction of microorganisms into the milling process seems to be dominated by microorganisms on the kernel husk (Gilbert 2010). However, the composition of the microbiota of the grinding material can change during the milling process. The total aerobic bacterial counts in the flour are therefore the result of both the bacterial input and process.

3.1.3.3 Bacterial counts in flour

The German Society for Hygiene and Microbiology has published guideline and warning values for cereal flours made from wheat, rye and spelt. The standard value for the aerobic mesophilic colony count (30 °C) is $2x10^6$ CFU per g. *For Escherichia coli*, a guideline value of $1x10^1$ CFU per g and a warning value of $1x10^2$ CFU per g apply; *Salmonella* must not be detectable in 25 g (DGHM 2015).

Enterobacteriacae like *Serratia, Pantoea, Escherichia, Enterobacter* and *Raoutella* are the predominant genera of the flour microbiota. In addition, other Gram-negative genera such as *Pseudomonas* or *Aeromonas* have been found (Gill, Carrillo et al. 2019).
The total aerobic bacterial count of flour has been investigated in several studies and mostly ranged between 100 and 10,000 CFU per g. *E. coli* are usually not detectable or only in very limited numbers (see Table 1).

Flour samples from various German mills examined by Mäde et al. contained *E. coli* in quantities of up to 200 bacteria per g in individual cases, correlating with the detection rates of the STEC found (Mäde, Geuthner et al. 2017, Mäde, Geuthner et al. 2018).

Table 1. Bacterial numbers detected in wheat flour from literature

Microorganism	Samples investigated	Counts / log CFU per g	Country	Reference
Bacteria	1	2.4 ± 0.1; *E. coli* not detectable	USA	(Suehr, Anderson et al. 2019)
E. coli	300	0.8	Australia	(Eglezos 2010)
APC	100	4.2		
E. coli	545	0.16-0.84	USA	(Sperber and Group 2007)
APC	435	4.41		
Aerobic plate count	24	3.32 to 4.25	Canada	(Gill, Carrillo et al. 2019)
APC	24	2.25-2.95		
E. coli	24	Not detectable		

APC: aerobic bacterial plate count; CFU: colony-forming units; MPN: Most probable number

3.1.3.4 Occurrence of STEC in flour

3.1.3.4.1 Outbreaks caused by STEC

There are reports of STEC outbreaks in the USA and Canada linked to flour (or raw cookie dough) (see Table 2).

Table 2: Overview of STEC outbreaks associated with flour or dough

Country/year	Food	Serotype	Number of disease cases	Reference
USA/2009 30 states	'ready-to-bake cookie dough'	O157:H7		CDC 2009, (Neil, Biggerstaff et al. 2012)
USA/2016 24 states	Flour (dough)	O121 and O26		CDC 2016, (Crowe, Bottichio et al. 2017)
USA/2016 9 states	Baking mix, (dessert pizza)	O157:H7		(Gieraltowski, Schwensohn et al. 2017)
Canada/2016–2017 9 provinces	Flour	O121:H19		(Morton, Cheng et al. 2017); CFIA 2017a
Canada/2017 1 province	Flour	O121		CFIA 2017b
USA/2019 9 states	Flour	O26		CDC 2019

Three children, in three different US states, who had received raw dough to play with fell ill with STEC O121 and O26 during the STEC outbreak in 2016 (Crowe, Bottichio et al. 2017). During the investigations, no source of contamination could be found in the factory. This might indicate a contamination of incoming raw material. In total, 250 different products containing flour were recalled (CDC 2016).

In another STEC outbreak in the USA in 2013 with serotype O121, frozen snacks were identified as a vehicle. Flour was suspected to be the cause, but no proof could be provided (CDC 2013).

The amount of STEC in the flour causing an outbreak in nine provinces of Canada has been determined to be between 0.15 and 0.43 probable bacterial counts per 100 g using a semi-quantitative statistical procedure (Most Probable Number; MPN). Additionally, the total aerobic bacterial count was determined to be between 4.48 and 4.79 log CFU per g. No correlation between coliforms (2.25-2.96 log CFU per g) and STEC recovery was found. The authors point out that the low STEC count requires a statistically validated random sample to reliably detect

STEC in flour or to establish its absence with a high statistical certainty (Gill, Carrillo et al. 2019).

Data from the USA indicate a prevalence of 9 % STEC-positive flour samples for the period 2010 to 2017 (Gonzalez-Escalona and Kase 2019). A more precise characterisation of STEC isolates from flour, including the outbreak strains O26 and O121, showed the following serotypes and virulence genetics: O26:H11 (*stx*1a, *eae*), O121:H19 (*stx*2a, *eae*), O111:H8 (*stx*1a, *stx*2a, *eae*), O103:H11 (*stx*1a, *eae*), O8:H14 (*stx*2d/e), O159:H9 (*stx*2d/e) and O103:H2 (*stx*1a, *eae*).

3.1.3.4.2 STEC in flour in Germany, Austria and Switzerland

Fifty-one samples, each consisting of 25 g wheat or rye flour, were analysed in Saxony-Anhalt, between 2014 and 2017 and the method for STEC detection and isolation from flour was optimised (Mäde, Geuthner et al. 2017). It was shown that more than fifty bacterial colonies have to be tested for a successful isolation. The investigated flour samples were all taken from mills that were also checked for their hygienic condition. In total, ninety-eight subsamples were analysed. In 38 of these samples, a molecular-biological detection of STEC was successful (39 %) and in 17 of 88 samples (one sample with 10 subsamples was not examined further), STEC could be isolated (19 %). Statistical analysis of different parameters showed no significant differences among the grain varieties but an increased detection rate with an increased number of subsamples. No sources of contamination could be identified. However, two possibilities are considered. In the first, an introduction of STEC by contaminated raw material (Gurtler, Keller et al. 2019) and multiplication during conditioning/tempering that could then establish and spread through flour residues on the equipment. In the second, a contamination of the flour within the mill is conceivable, e.g. via contaminated water or droppings of rodents or birds. Recent data show a correlation between the STEC detection and the sampled mills and identify the tempering of the cereal grains as a critical step in the production, since water is added and microorganisms can multiply during the duration of conditioning in the tempering cell at room temperature. STEC were detected in the investigated residues. This could potentially lead to contamination of the flour during the milling of the cereal grains (Mäde, Geuthner et al. 2018).

Fifty STEC-positive samples (15.2 %) were detected in 328 wheat, spelt and rye flour samples analysed in Germany in 2018 (BVL 2019) as part of the Federal Monitoring Plan (BüP). In the same period, cereal grass products were also analysed and only one of 243 samples was STEC positive (0.4 %). Furthermore, a product recall (Nov. 2019) of ready-made dough for short pastry biscuits (source: RASFF 2019.4057) shows that STEC can also occur in such a matrix.

In the last four years (01/2015-09/2019), the National Reference Laboratory (NRL) for *E. coli* has received 133 STEC isolates from flour (including those from BüP 2018 and isolates from Mäde et al. 2017), baking mixes and cereal waste for analysis. The preliminary STEC results could not be confirmed for six isolates (see Table 3 and 4).
Of the 133 STEC isolates, 28 were considered duplicates, i.e. the same STEC serotype with the same virulence factors was presumably isolated more than once from the same sample

(same sample number of the sender). Duplicates are not included below and only 105 STEC isolates are considered.

The 105 single STEC isolates were derived from different types of wheat (n=62), rye (n=27) and spelt flour (n=5). The most common matrix was wheat flour type 550 (n=36). Additionally, four isolates from baking mixtures (2x whole meal bread, 1x biscuit, 1x muffin) and one isolate from cereal waste have been analysed. The isolates were determined to comprise 27 different serotypes. Most frequent were isolates of the serotypes O187:[H28] (19 %, n=20), O154:[H31] (13 %, n=14), O11:[H48] (10 %, n=10) and O36:[H14] (10 %, n=10) from flour, baking mix and cereal waste. Serotypes which had been seen in samples of human disease cases were isolated from rye and wheat flours and from baking mixes and cereal waste. These include the serotypes O8: [H9/H19], O79: [H14], O103: [H3], O117: [H4], O127: [H12], O145: [H25], O146: [H25], O156: [H25], O157: [H7], O187: [H28]. Serotypes O157:H7, O103:H2 and O145:H28, which are considered highly virulent and often associated with HUS, were isolated from various rye, wheat and other cereal flours (see Table 3). In total, 40 % of the isolates from rye flours (11 of 27) and wheat flours (25 of 62) belonged to clinically relevant serotypes. The isolates of serotype O157:[H7] belonged to the stx2c subtype, associated with (more severe) diseases. In the subtyping of the Shiga toxin genes, *stx*1a (7x), *stx*1c (1x), *stx*1d (37x), *stx*2b (15x), *stx*2c (2x), *stx*2e (3x) and *stx*2g (35x) were found. The types *stx*2a, *stx*2d, *stx*2f and the combination of *stx*1 and *stx*2 were not detected.

In addition to one of the *stx* subtypes, the gene (e*ae*A) for the adhesion factor intimin, which is considered to be another important virulence factor, was detected in nine strains.

Table 3: Properties of the STEC isolates from flour samples characterised at the NRL *E. coli*

Origin (N)	Matrix information[1] (N)	Serotypes
Flour (94)	Spelt flour	
	Spelt flour, type 630	2x O154:[H31]
	Spelt flour, type 1050 (1)	O36:[H14]
	Wholemeal spelt flour (1)	O36:[H14]
	No further information (1)	ONT:[H31]
	Rye flour (27)	
	Rye flour, type 1150 (11)	**O8**:[H9], O11:[H48], O12:[H45], O21:[H21], O36:[H14], 2x O154:[H31], **O157**:[H7], O175:[H28], 2x O187:[H28]
	Rye flour, type 997	O12:[H45], O43:[H2], O156:[H25], O175:[H28], 2x O187:[H28]
	Wholemeal rye flour	**O8**:[H19], O36:[H14], O79:[H14], O110:[H31], O179:[H31], O187:[H28]
	No further information (4)	O11:[H48], O156:[H25], ONT:[H7], ONT:[H23]
	Wheat flour (62)	
	Wheat flour, type 405 (11)	**O8**:[H19], O11:[H48], **O103**:[H2], O117:[H4], **O145**:[H28], 2x O154:[H31], O175:[H28], O187:[H28], ONT:[H23], ONT:[H31]
	Wheat flour, type 550 (36)	2x **O8**:[H19], 3x O11:[H48], 4x O21:[H21], 4x O36:[H14], 2x O43:[H2], O127:[H12], **O146**:[H28], 5x O154:[H31], O156:[H25], O175:[H7], O175:[H28], 9x O187:[H28], ONT:[H23], Or:[H23]
	Wheat flour, type 812 (1)	O12:[H45]
	Wheat flour, type 1050 (3)	**O157**:[H7], O175:[H28], O187:[H28]
	Wholemeal wheat flour	**O8**:[H9], O11:[H48]
	No further information (9)	2x O11:[H48], O36:[H14], 2x**O146**:[H28], O154:[H31], O156:[H25], O175:[H28], ONT:[H19]
Other (11)	Mixed flours[2]	**O8**:[H19], O11:[H48], O79:[H23], **O103**:[H2], O154:[H31], O187:[H28]
	Baking mixes[3] (4)	O36:[H14], O154:[H31], 2xO187:[H28]
	Cereals/cereal waste (1)	O187:[H28]

1) Matrix information from sender (ADV matrix code L); 2) 'Cereal flours and flours from other cereal grains'; 3) Biscuit (1), wholemeal bread, muffin baking mix (1);
[H]: molecular definition of H-type
bold = serogroups which have frequently been detected in human disease cases (RKI 2019); **underlined** = serotypes which have frequently been described in severe clinical disease courses in humans (Mellmann, Bielaszewska et al. 2008)

Within the frame of a research project, the genomes of selected strains have been sequenced. The data from two isolates of serotype O146:[H28], one O103:[H2] isolate and one O157:[H7] isolate have been compared with a sequence database of human associated STEC strains at the Robert Koch-Institute. A comparison of 2055 gene-loci/alleles resulted in the grouping of the O157:[H7] food isolate with a human isolate having only 8 alleles difference (cluster distance thresholds of 10 alleles, E. coli MLST Warwick).

One of the six non-STEC isolates examined (BfR-EC-17761) could be assigned to serotype O121:[H19] (see Table 4). Strains of this serotype have been responsible for two flour associated outbreaks (see Table 2) and STEC of this serotype usually possess the virulence factors

eae and *nle*B (Bugarel, Martin et al. 2011). The analysed isolate from wheat flour also possesses the virulence factors *eae* and *nle*B. This isolate is therefore to be classified as enteropathogenic *E. coli* (EPEC).

Table 4: Overview of non-STEC isolates investigated

Description	Matrix information[1]	Serotypes, virulence factors	Previous finding[2]
	Wheat flour, type 550	O11:[H5], *ehxA*	VTEC
	Wheat flour, type 550	O121:[H19], *eae*, *nleB*	VTEC
	Wheat flour, type 1050	Not determined	EHEC
	Rye flour, type 1150	O150:[H8]	VTEC
	Rye flour, type 1150	O7:[H6]	VTEC
	Rye flour, type 1150	O8:[H8]	VTEC

1) Matrix information from sender (ADV matrix code L); 2) Previous finding reported

Recent data from Switzerland show an STEC prevalence of 10.8 % in 93 flour samples of various cereal varieties (wheat and wheat mixtures with other cereal flours) from retail outlets (cantons of Berne and Aargau; origin Switzerland and others including Germany). The isolates obtained have been typed using whole genome sequencing and examined for virulence-associated genes (Boss and Hummerjohann 2019). Among the STEC strains obtained, one with the *stx2* gene (*stx2*a) and *eae* from serogroup (O26) was detected. This group is often associated with outbreaks. The other strains display different serotypes and a large diversity in their virulence gene pool, but were not assessed as high risk (Kindle, Nuesch-Inderbinen et al. 2019).
Data from Austria have shown positive signals for Shiga toxin genes (5x *stx2*g, 1x stx2b) in six (19.3 %) of 31 flour samples of different cereals from nine mills. Four samples were also culturally positive, but STEC could only be isolated from one culture (O36:Hrough). From two other samples, two more STEC isolates (one from each sample) were obtained after ten months of storage at room temperature (both Orough:H28). The background microbiota had probably been sufficiently reduced during storage. A comparison with human isolates has shown no similarities (Schlager, Schlagenhaufen et al. 2018).
Taking into account the methodological differences in the different studies, it can be concluded that the prevalence of STEC in flour must be in general between 10 % and 30 %. The extent to which the different prevalences observed here are due to methodological differences (different bacterial counts or technological differences in germ reductions) cannot be estimated at present and should be the subject of further investigation.

3.1.3.5 Survival of STEC in flour

Various studies have shown the survival of STEC over several months in dry foods such as walnut kernels, infant rice cereal, apple powder and buttermilk powder (Beuchat, Komitopoulou et al. 2013, Burgess, Gianotti et al. 2016).
Storage tests for STEC of serogroups O26, O103, O111 and O157 in flour (a_W 0,44) have shown that all tested serogroups remained viable at room temperature (23 °C) for at least nine months. Strains of serotype O157:H7 were still detectable even after one year. A data set for

storage at 35 °C has shown that no STEC was quantifiable after seven days and that they were no longer detectable after 49 days even after enrichment (initial bacterial count 8 log CFU per g). For products made of flour that cannot be heated (e.g. for certain cakes), the authors recommend storing the final product for two months at a slightly elevated temperature (35-40 °C), as Salmonella and STEC would both be inactivated (Forghani, den Bakker et al. 2018). The inactivation kinetics at temperatures of 55, 60, 65, and 70 °C for the same strains (see Table 5) have shown that they survived for up to 60 min (endpoint of experiment) at 70 °C in wheat flour. D-values have been calculated to be between 5.75 min and 8.67 min depending on the serogroup. For comparison, the D-value for *Salmonella* Enteritidis in flour at 75 °C was 9.97 min (Smith, Hildebrandt et al. 2016). For strains of serogroups O121, O145 and O45, a decimal reduction at 55 °C for 20.0 - 49.3 min, at 60 °C for 4.9 - 10.0 min, at 65 °C for 2.4 - 3.2 min and at 70 °C for 0.2 - 1.6 min could be determined. All investigated STEC strains were directly quantifiable at room temperature (23 °C) for 84 days and were still detectable with enrichment at the end of the experiment after 168 days (Forghani, den Bakker et al. 2019).
A comparison of the outbreak strains O121 (USA, 2016) and O157 (USA, 2009) with respect to their survivability during drying and storage has shown a significantly higher survivability for O121:H19 after 24 hours compared to O157:H7. However, both were still detectable at the end of the experiment after seven days (Suehr, Anderson et al. 2019).
Experiments on heat inactivation of STEC in flour for a strain of serotype O157:H7 isolated from cookie dough have shown a 5 log reduction of the *E. coli* O157:H7 population after the application of indirect dry heat for 5 minutes at 70 °C or for 15 minutes at 65 °C. However, viable bacteria could still be detected after 30 minutes (endpoint of experiment) at 70 °C (Greene 2012). A comparison of the data on heat inactivation is not straightforward , as it has been shown that different cultivation methods (e.g. cultivation of the bacteria in liquid or solid culture media, distribution in the foodstuff flour, heating processes etc.) can lead to different results (Hildebrandt, Marks et al. 2016).
It should be also considered that heat inactivation of STEC in foods with low water content usually requires higher temperatures and is partly dependent on the bacterial strain (Beuchat, Komitopoulou et al. 2013, Forghani, den Bakker et al. 2018). In a recent study STEC was stored at 6 °C in flour with a moisture content of 13 % (a_W 0.55) and subsequently treated at 82°C for five minutes. A reduction of more than five log units was observed and the STEC were no longer detectable. For comparison, in flour with 8 % moisture (a_W 0.25) the same temperature and time combination led to a reduction of only two log units and was not sufficient to completely inactivate STEC. Outbreak-associated STEC strains have shown increased survivability. Surrogate bacteria such as *E. coli* K12 are often used for process validation purposes. When this strain was tested with STEC in parallel, its survival rate in flour and heat tolerance was, on average, lower than that of the investigated STEC strains (Daryaei 2019).
(Wheat) flour has been used in several studies as a substrate for heat inactivation experiments for various parameters, including other human pathogens such as *Salmonella* (Smith, Hildebrandt et al. 2016, Syamaladevi 2016).
It is generally reported that during drying and storage bacteria can enter a dormant state from which they can recover by rehydration (Scherber, Schottel et al. 2009). This was also suspected for the pathogen on the fenugreek seed in the sprout-associated *E. coli* O104:H4 outbreak in 2011 (Aurass, Prager et al. 2011).

Table 5. Survivability of STEC in flour at various temperatures

Food	a_W value	Serogroup	Temperature [°C]	D-value [min]	Sigma value [min] (Weibull)*	Reference
Wheat flour	0.48	O26, O103, O111, O157	55	46.53 - 61.10	26.6 - 15.56	(Forghani, den Bakker et al. 2018)
			60	11.24 - 16.10	5.07 - 9.24	
			65	8.01 - 12.20	2.96 - 3.95	
			70	5.75 - 8.67	0.14 - 1.74	
Wheat flour	0.45	O45, O145, O121	55	N/K	20.0 - 42.9	(Forghani, den Bakker et al. 2019)
			60	N/K	4.9 - 10.0	
			65	N/K	2.4 - 3.2	
			70	N/K	0.2 - 1.6	
Wheat flour	0.45	O121 (outbreak strain)	70	18.2 ± 1.0	4 ± 1	(Suehr, Anderson et al. 2019)
			75	6.5 ± 0.5	0.7 ± 0.5	
			80	4.6 ± 0.4	3 ± 1	

D-value/Sigma value: Periods when a given temperature is required in order to reduce a microorganism population to 10 %.
*The mathematical formula for the Weibull model contains time and route-dependent parameters, meaning that data sets with different populations at the beginning cannot be compared with each other! More recent studies favour the Weibull model, on the basis that the determination coefficient is generally better (Greene 2012, Forghani, den Bakker et al. 2018).

For *E. coli* O157:H7, reductions of the bacterial count in cookie dough (a_W = 0.8; high sugar and fat content) by 3 log units at 4 °C and 2.7 log units at -20 °C were described. However, detection of *E. coli* O157:H7 was still possible after eight weeks (Wu, Ricke et al. 2017). Significant effects on the survival rate at 4 °C were demonstrated in doughs with compositions modified by increasing the sugar content and adding butter as a fat component (compared to margarine, coconut oil and olive oil). Nevertheless, under all conditions, *E. coli* O157:H7 (initial concentration 5.5 log CFU per g) was still detectable after 8 weeks. The authors did not investigate the growth kinetics of the bacteria (Wu, Ricke et al. 2017).
Studies on the heat inactivation of STEC during the baking process are not available. *Salmonella* (*S*. Senftenberg 775W ATCC 43845, *S*. Typhimurium ATCC 14028 and *S*. Newport ATCC 6962), were introduced into Hamburger roll dough (a_W 0.97) and muffin baking dough (a_W 0.92) by artificially contaminated flour. The application of the baking process (≥ 218.3 °C or 190.6 °C) with a necessary baking time of at least 9 minutes and 17 minutes, respectively, reduced the bacterial count by 5-6 log units (Channaiah, Holmgren et al. 2016, Channaiah, Michael et al. 2017). At a core temperature of 75-85 °C, a reduction by five log units was achieved within 6 minutes for Hamburger rolls and for muffins (core temperature 90-100 °C), within 17 minutes.
Gieraltowski and colleagues discuss a contamination of the dessert ("dessert pizza") identified in a O157:H7 outbreak either by the dusting of surfaces with flour to transfer the pizza or via thicker areas of the dough, which may not have been baked sufficiently to the core (Gieraltowski, Schwensohn et al. 2017).

3.1.3.6 Consumption of flour and flour products

Preliminary data for the 2016/2017 harvest year show a per capita consumption of cereal products of 69 kilograms (kg) per year in the hard and soft wheat flour category and an additional 7.2 kg per year for rye flour (BMEL; 2019). These data have not been specified for product groups (bakery products, pasta, flour direct etc.). According to the statistical yearbook of the

BMEL, there are 205 mills in Germany that are subject to reporting requirements (BMEL; 2019). According to the German Milling Association, the average mill supplies 400,000 people with ground products every day. The German Milling Association estimates that ≤10 % of all flour is distributed by retailers and consumed in private households (www.muehlen.org, (BMEL; 2019)). All population groups, including the particularly sensitive groups (infants, the elderly, pregnant women and immunocompromised persons) consume flour based products on a regular basis.
Flour is normally not consumed directly and is usually heated before consumption (as in baked goods or similar). In exceptional cases, flour products, e.g. raw cookie dough, are consumed without a heating step. These raw cookie doughs are seen as a trend food and are advertised accordingly in Germany (example: https://www.groupon.de/deals/spooning-cookie-dough-keksteig). In a survey on the consumption of risky foods in the USA, 53 % of the young adults questioned stated that they consume raw cookie dough prepared at home (Byrd-Bredbenner, Abbot et al. 2008). Data on raw cookie dough consumption in other countries, including Germany, are not available. However, there is also no evidence of a significantly different consumer behaviour in Europe. Particularly in the case of small children, it has to be expected that dough is consumed raw in small quantities due to tasting or licking of the fingers during baking in the households, in community facilities or around the campfire. In exceptional cases, it is also possible that baked goods are consumed even though they are not baked through.
In addition, all population groups may ingest raw flour as a separating or binding agent and with products flour dusted after baking.
In general, flour is a stable foodstuff, with a stated minimum shelf life for fine flours of between one and one and a half years (http://www.mindesthaltbarkeitsdatum.de/haltbarkeit-von-lebensmittel/haltbarkeit-von-mehl/). The long shelf life leads to a wide distribution of manufacturing batches on retail shelves and in private households. This makes it difficult to trace the products in cases such as product recalls (Neil, Biggerstaff et al. 2012, Gieraltowski, Schwensohn et al. 2017).

3.1.4 Risk characterisation

Flour is a minimally processed natural foodstuff that is not usually subject to a bacterial reduction step. The shelf life of the flour depends on the degree of milling and the composition (type) and is, for example, between one and one and a half years for type 405 wheat flour. Thicker milled flours and darker flours have a shorter shelf life (6-8 months). Flour is widely distributed and is a component of countless products.
STEC contamination of the grain can occur during cultivation, harvest, transport or processing (in the mill), and thus these pathogens can be introduced into the flour and blended in by subsequent process steps. Possible sources of contamination include soil and irrigation water and also wildlife. The total aerobic bacterial counts found in flour range between 10 and 10,000 CFU per g though *E. coli* as a hygiene parameter is detected only rarely. Investigations of flour samples from mills in Germany on the presence of STEC have shown between 10 and 21 % positive samples. The detection rate depends on both the number of samples examined and the sample volume. From samples that had shown a positive result in a molecular-biological test, the subsequent isolation of STEC was only successful in 50% of the cases.

Methodological challenges exist in detection and isolation, since the bacteria are present in small numbers and very heterogeneously distributed and may also be present in a dormant state. In addition, the background microbiota of the flour makes detection and isolation difficult. The typing of the STEC isolates found in Germany shows a large number of different serotypes, including clinically relevant serotypes, and the presence of different *stx* types and other virulence genes.
Viable STEC of different serogroups are detectable in flour over more than 50 weeks. They show a high heat stability, in some cases surviving significantly longer than five minutes at 70 °C, though this heat stability depends on the moisture content and also varies somewhat according the method and data evaluation used (linear regression vs. Weibull model). The high heat stability is attributed to the low a_W-value, about 0.4 to 0.45, of flour. Experiments with dough (a_W value 0.8 in the referenced example) show limited dependence on the dough ingredients (sugar, fat) for the survival of the STEC investigated. STEC multiply at a_W-values higher than 0.95. For a risk assessment, further data are required on the behaviour of STEC in different doughs under different conditions.
Flour products are consumed regularly by all population groups, including the particularly sensitive groups, but usually after heating. In rare cases, raw dough and incompletely baked products may also be consumed, especially by children. In addition, all population groups may ingest raw flour as a separating or binding agent and with products flour dusted after baking.

There are reports of flour-associated outbreaks in the USA and Canada, often involving the consumption of raw dough or describing raw dough as a toy. No EHEC outbreaks attributable to flour or flour based products have been reported so far in Germany. Although not yet directly demonstrated in Germany, the highly reliable match of a flour isolate with a human isolate (EHEC O157:H7), shows that the presence of highly pathogenic EHEC in flour here is a possibility. Cookie dough and cookie dough base mixes for raw consumption are manufactured and distributed in Germany with pasteurised flour. However, little is known about the exact parameters and technology of the heat treatment and it is unclear whether proof of the effectiveness of the heat treatment can always be provided. Dry heat requires a much more intense application to attain the same efficiency in bacterial inactivation as that achieved in the pasteurization of liquid products. For a conclusive evaluation of commercially distributed products, an investigation of the usual treatment methods is necessary.
The recommendation of heat treatment of a foodstuff for two minutes at a core temperature of 70 °C is not sufficient to safely kill STEC in flour using dry heat.

The following scenarios are intended to assess the risk posed by STEC in flour.

Scenario 1: Flours, baking mixes and raw dough pieces not intended for retail sale

It is possible that STEC-contaminated flours are introduced into the production of baking mixes and raw dough pieces not intended for retail sale.

The risk for the operators in the manufacturing facility is regarded as very low if the general hygiene rules are applied and spreading of flour dust is avoided.

The risk for consumers is considered negligible if the flour or raw dough pieces are heat treated in a validated baking step and if spreading of flour dust is avoided.

There is no evidence for a risk derived from the professional use and handling of flour or raw dough pieces based on the available data. Future scientific findings could change this assessment. However, the risk of an EHEC infection through consumption of bakery products increases if the bakery products are dusted with flour after baking.

Scenario 2: Flours, baking mixes and ready-made doughs which are sold in retail

It is possible that STEC-contaminated flours, baking mixes and ready-made doughs are sold in retail outlets.

If the flour is processed into a dough and then baked in the oven according to recipe/manufacturer specifications, the risk for consumers of an EHEC infection due to the consumption of these baked products is regarded as very low. Nevertheless, there may be risks from flour dusts and cross-contamination, which can be minimized but not completely eliminated by good kitchen hygiene.
Flour, unlike raw eggs or raw meat, is not generally recognised by the population in Germany as a potential source of contamination. This point is of particular importance. The storage of prepared dough should be as brief as possible and preferably refrigerated
Despite the extensive use of heat-treated flour in ready-made doughs, it cannot be ruled out that doughs made from untreated flour which is contaminated with STEC may enter retail. As the parameters and technology used for heat treatments by individual manufacturers are not known to the BfR, it is also not possible to assess the effectiveness of heat treatments.
If the finished dough is baked in the oven according to recipe/producer specifications, the risk to consumers is considered negligible. Possible cross-contaminations can be minimized by good kitchen hygiene.
The consumption of raw dough poses a risk of EHEC infection for all population groups. However (young) children are especially vulnerable in terms of infectious risk and severity of a possible disease.
Further statements are not currently possible due to the lack of valid data on the behaviour of STEC in different types of dough (yeast dough, wafer dough, bread roll dough, ...) under different conditions (time, storage temperature, yeast fermentation).
New scientific findings could change this assessment.

The STEC infection risk for consumers after consumption of raw or insufficiently heated flour products contaminated with STEC depends on the type and quantity of bacteria ingested and the susceptibility of consumers to these pathogens.
Since STEC can survive very well in flour and the infectious dose is very low, the risk of infection exists independently of further bacterial propagation in the food. In healthy adults, an infection would probably cause a mild to severe diarrhea with hospitalisation in individual cases. The infection can cause a hemolytic-uremic syndrome (HUS) especially in small children. The HUS is associated with bloody diarrhea and kidney failure, can cause permanent dialysis dependency and, in individual cases, even death.
According to current knowledge, baking flour products according to standard recipes and manufacturers specifications is suitable for killing the bacteria. Further data are required on the

behaviour of STEC during the baking process at different compositions of the baked goods and different temperatures.

3.1.4.1 Evaluation of the quality of the data

The quality of the data available and information concerning the properties of STEC, their transmission to humans and the diseases caused by these pathogens is considered satisfactory. The quality of data on the distribution of STEC in flour and its heat stability is considered generally satisfactory, but there is a lack of data on distribution by type of cereal, cultivation/management (organic, conventional), area of cultivation, irrigation, plot size, associated livestock, wildlife, etc. To a lesser extent, data on pathways of entry into cereals and flour are available and allow an estimation of the probability of STEC being present in these matrices. However, data on the effectiveness of decontamination measures in mills or on the behaviour of the pathogens in different doughs are insufficient.

3.1.4.2 Need for further research

There is a need for research on the following questions:

- Pathways of pathogenic microorganisms into cereals and flour
    - Dependence on the grain used (wheat, rye, spelt etc.)
    - Dependence on primary production (cultivation [organic, conventional], cultivation area, irrigation, plot size, associated animal husbandry, occurrence of game etc.)
    - Further contamination routes (water, personnel, insects, birds, rodents etc.)
- Prevalences of STEC in flours and flour-containing products depending on the type of flour and origin, production areas and transport routes
- Effectiveness of risk mitigation measures in the mill (prevalence in production environment, efficiency of cleaning and, if necessary, disinfection measures)
- Behaviour of pathogenic microorganisms and the microbiome in different types of dough (yeast dough, wafer dough, bread roll dough, ...) as a function of temperature and time
- Optimisation and standardisation of the methods for isolating STEC from flour and flour products (standardisation of detection is particularly important for comparing prevalences).
- Importance of dormant cells for diagnostics, research on the revival of dormant cells and/or detection of these cell stages
- Effectiveness of the applied heat treatment technologies to flour in killing STEC
- Determination of the prevalence of STEC in flour, taking into account methodological differences, bacterial introduction and technology related bacterial reduction
- Investigation of alternative measures for inactivating STEC in flour, e.g. conditioning of grain with hot steam, treatment of grain with ozone or UV radiation, irradiation of finished doughs, with regard to effectiveness and influence on nutritional aspects as well as consumer acceptance

3.2 Further aspects

The standard method ISO/TS 13136:2012 (under revision) "Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and determination of the serogroups O157, O111, O26, O103 and O145" has been modified by Mäde et al. to improve the detection of STEC in flour and in particular to make the isolation of STEC from flour samples more effective.
The National Reference Laboratories in Europe receive flour samples artificially contaminated with STEC from the European Reference Laboratory (for the EU-RL proficiency test 25).The samples are used for general verification of the methods in the framework of the bi-annual inter-laboratory comparison tests.
To estimate the prevalence of STEC in flour (at mill level) in Germany, it was suggested to include this matrix in the zoonosis monitoring 2020.

3.3 Framework for action / measures

The microbiological status of finished doughs is unknown. The microbiological status is influenced by a complex mixture of factors such as bacterial contamination through raw materials, e.g. flour, and specific bacterial reduction measures applied by the manufacturers. Therefore, the BfR suggests including the product group of ready-made doughs in the national monitoring programs BüP and/or zoonosis monitoring.

The BfR recommends the following risk mitigation measures to manufacturers of flours, baking mixtures and ready-made doughs:

1. Evaluation of the effectiveness of cleaning and disinfection plans (where applicable) on a regular basis via suitable self-monitoring programs. The programs should include tests on samples for the presence of STEC. An action plan must be ready for implementation when STEC is detected within the internal control program.
2. Heat treatment of ready-made dough for retail sale or production of ready-made dough from heat-treated flour (effectiveness of the heat treatment steps used should be demonstrated, including the reliable killing of STEC)
3. Ready-made doughs must not contain pathogenic bacteria.
4. Avoid and thoroughly remove flour dusts wherever possible.
5. Avoid dusting of baked goods with flour after baking.

Further risk mitigation measures, such as conditioning the grain with hot steam, treating the grain with ozone or UV radiation and irradiating ready-made doughs, still require technological development and research with regard to effectiveness and influence on nutritional aspects as well as consumer acceptance.

The BfR recommends consumers to protect themselves against food-borne infections by:

1. Always storing and processing flour, baking mixes and raw dough separately from other foodstuffs.
2. Processing doughs as quickly as possible or storing them in the refrigerator.
3. Consuming doughs and baked goods only after complete heating.
4. Cleaning kitchen utensils and surfaces thoroughly after contact with flour, baking mixtures or raw dough.
5. Washing and drying hands thoroughly after processing flour, baking mixes or raw dough.

To make the German public aware of the possible presence of pathogens in flour, existing consumer guidance should be amended accordingly. Facilities with a multiplier function and particularly vulnerable groups of people (community facilities such as schools and day-care centres) should also receive appropriate informational material.

Further information on the BfR website on the topic...

BfR opinion on Shiga toxin-producing *E.coli* in foods: Prediction of the disease-causing potential of the various strains not yet possible

https://www.bfr.bund.de/cm/349/shiga-toxin-producing-e-coli-in-food.pdf

BfR opinion on the EHEC outbreak of 2011: Updated Analysis as a Basis for Recommended Measures

https://www.bfr.bund.de/cm/349/ehec-outbreak-2011-updated-analysis-as-a-basis-for-recommended-measures.pdf

BfR 'Opinions app'

4. References

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