GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

Bundesinstitut für Risikobewertung

# STEC in dough and batter

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* BSc-Thesis: Studies on *E. coli* strains including STEC in liquid dough; **BSc-Thesis: Considerations on STEC in ready-to-bake cookie dough: Heat tolerance and inactivation of Shiga toxin producing *E. coli* in flour-based products

# Background – Why dough and batter?


- STEC need $a_w$ > 0.95 for growth, can survive outside these conditions[1, 2, 3]
- STEC shown to survive in multitude of low $a_w$ food matrices (jerkies, cereals, nuts, milk powder, **flour**)

STEC in flour in Germany and Europe

| Timespan | Country | Samples | Detection-/Isolation rate | Ref |
|---|---|---|---|---|
| 2014 - 2017 | Ger (Saxony-Anhalt) | 98 | 39 % / 19 % | 4 |
| May – December 2018 | Ger<br>Federal control plan | 238 (85* + 153) | 14,4 % Isolation<br>(21,2 % or 10,5 %**) | 5 |
| 2020 | Ger<br>National Zoonosis Monitoring | 242 | 9,1% Isolation (95% CI 6,0-13,4) | 6 |
| July 2017 | AT | 31 | 19,4 % / 3,2 % or 9,6 % | 7 |
| October 2017 – March 2018 | Ch | 93 | 10,8 % / 8,6 % | 8 |
| March 2018 | Ch | 70 | 12,9 % / 11,4 % | 9 |

1) Beuchat et al., 2013 2) Forghani et al., 2018 3) Gill et al., 2020 4) Mäde et al. 2017; 5) BüP 2018; 6) Schlager et al 2018; 7) Boss et al. 2019; 8) Kindle et al. 2019; 9) National Zoonosis Monitoring 2020 (unpubl)

* Duplicate samples; **STEC-Detektion

# Background – Why dough and batter?

## STEC in flour / Outbreaks

| Year / Country | Matrix | Disease – Hospitalization – HUS | STEC-Type | Tracing | Ref |
|---|---|---|---|---|---|
| 2009 USA / 30 States | **Ready-to-bake cookie dough** | 77-35-10 | O157:H7 | Epidemiology | 1 |
| 2015-2016 / USA, 9 States | **Dough-Mix** (Desert Pizza) | 13-8-2 | O157:H7 | Epidemiology | 2 |
| 2016 / USA, 24 States | Flour (**Dough, Play - dough**) | 63-17-1 | O121; O26 | Laboratory (SNP) | 3, 4 |
| 2016-2017 / Canada | Flour | 30-8-1 | O121:H19 | Laboratory (PFGE / wgMLST) | 5 |
| 2019 / USA, 9 States | Flour | 21-3-0 | O26 | Laboratory (PFGE / wgMLST) | 6 |
| 2021 / USA, 12 States | **Dough Mix** | 16-7-1 | O121 | Laboratory (PFGE / wgMLST) | 7 |

1 Neil et al. 2012; 2 Gieraltowski et al. 2017; 3 CDC 2016; 4 Crowe et al. 2018; 5 Morton et al. 2017; 6 CDC 2019; 7 CDC 2021

# Questions –Dough and batter

1) What is the STEC-prevalence in ready-to-bake cookie dough?

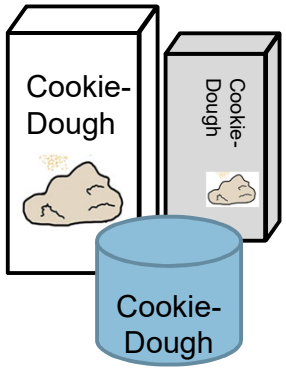

3) What happen to STEC during cookie baking?

2) How high is the contamination in ready-to-bake cookie dough?

4) What happen to STEC during pancake/waffel baking?

5) How long do STEC survive in dough and dough mixes?

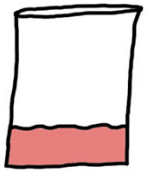
# STEC prevalence in ready-to-bake cookie dough




11 retail samples

November / December 2019

25 g

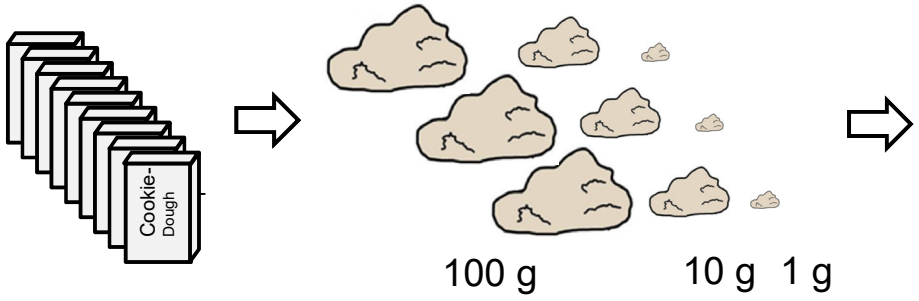


Analysis according to ISO/TS13136:2012 and §64 LFGB L25.00-6, 2017-10

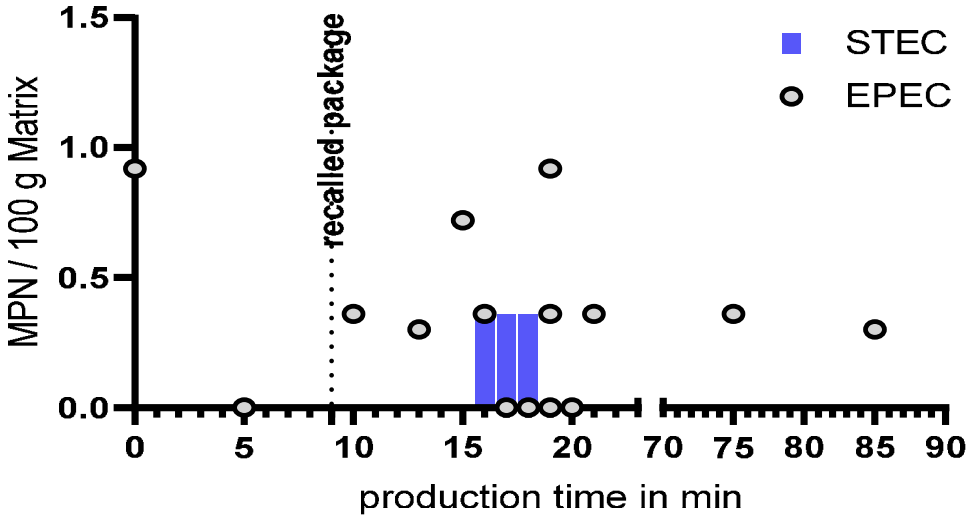
**No STEC detectable**

BUT: product recall of ready-to-bake cookie dough with spelt flour in December 2019

# STEC contamination level in ready-to-bake cookie dough




Analysis of 333 g with MPN




Batch of

22 packages

- 13 samples over the production time of 85 minutes
- plus 9 additional (together 10) samples after 19 minutes of production

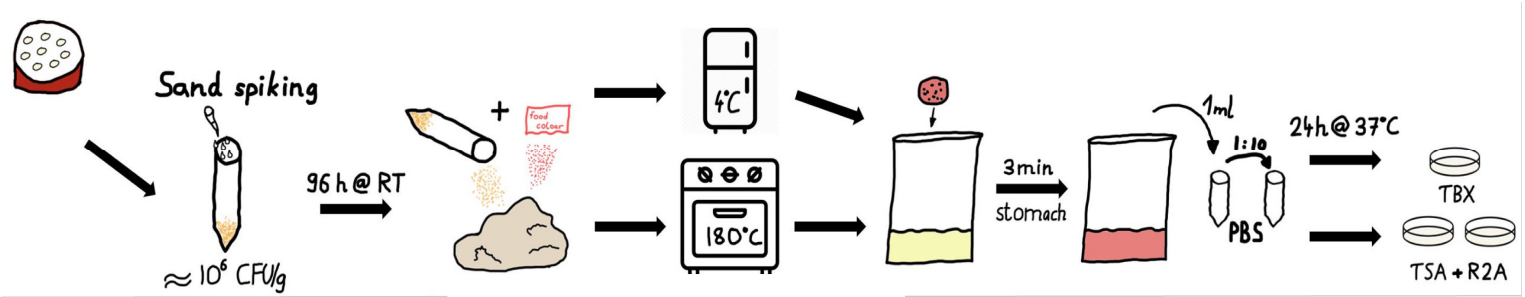
3 of 22 (13.6%) samples were STEC positive

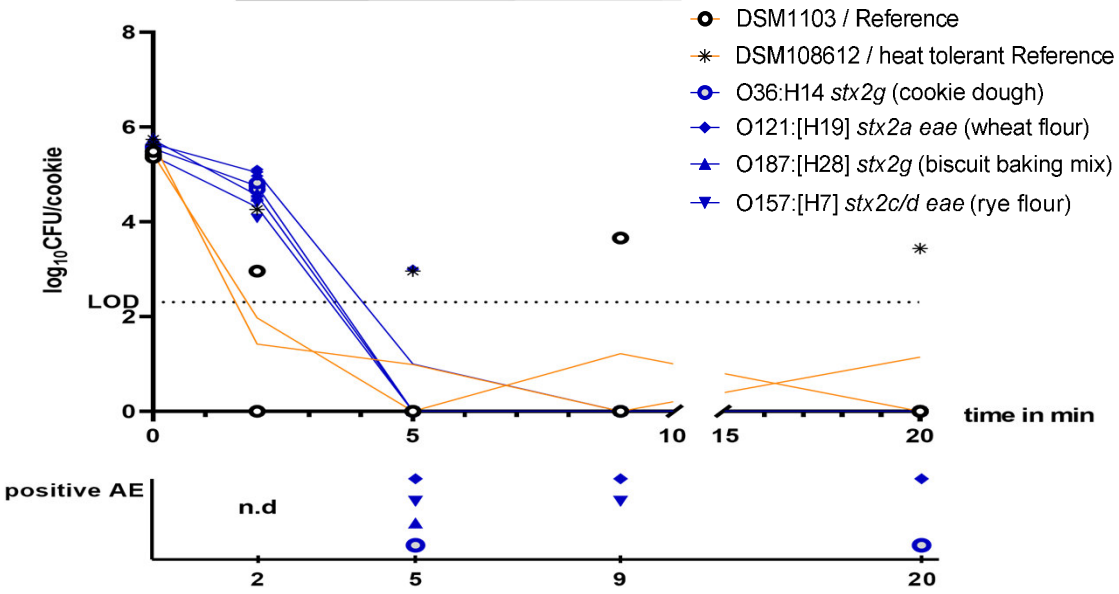
→ Range 0-0.36 MPN / 100 g

14 of 22 (63.6%) samples were EPEC positive

→ Range 0-0.92 MPN / 100 g

# STEC during cookie baking



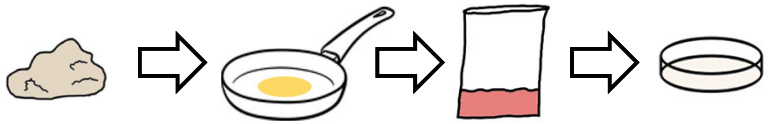

CFU development during baking of artificially spiked "ready-to-bake" cookie dough; Shown as mean (line) of indidividual biological replicates (dots); reference strains orange, STEC in blue; LOD Limit of detection ($10^{2.3}$ CFU / Cookie), n.d. not determined, AE Enrichment in Peptone water

→ inactivation of STEC slower than reference strains

→ no detection of STEC through direct plating after 5 min / all detectable after enrichment

→ reductions of ~ 3 log / no complete elimination of all strains after baking + cooling

# STEC during pancake/waffel baking



| dough | time [min] / strain | 0 | 1  | 2  | 3  | 4[1]  |
|---|---|---|---|---|---|---|
| Pancake mix | DSM1103 | ++ | + | + | + | - |
| | AW 1.7 | ++ | + | + | + | - |
| | EDL933 | ++ | + | + | ++[2] | - |
| | BfR-EC-17176 | ++ | + | + | + | - |
| Waffle mix (glutenfree) | DSM1103 | ++ | - | - | - | - |
| | AW 1.7 | ++ | ++ | + | - | - |
| | EDL933 | ++ | ++ | - | - | - |
| | BfR-EC-17176 | ++ | ++ | - | + | + |

1) Including flipping of pancake; 2) dough was lumpy; ++ Growth was detected after baking on TSA plates and after enrichment in peptone water on TBX plates; + Growth was only detected after enrichment on TBX plates; - Growth was neither detected after baking on TSA plates nor after enrichment in peptone water on TBX plates

→ no detection of STEC through direct plating after 2 min / sporadic detectability after enrichment

→ complete elimination of 3 of 4 strains after baking (incl. flipping)

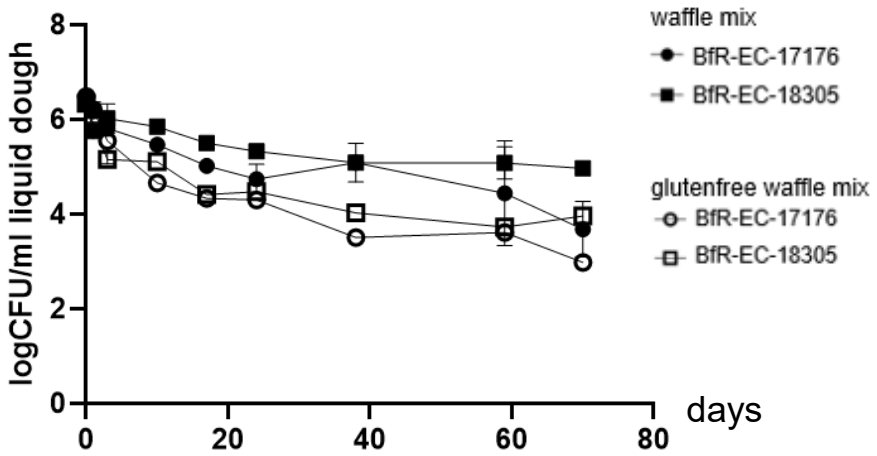
# STEC Survival (preliminary data)

Stability in Cookie Dough:

Detection of STEC after 6 month of storage (4°C-8°C) in ready-to-bake cookie dough ($a_w$ ~ 0,61-0,68 @ 23.8°C)

Stable STEC CFU in self produced dough over 4 weeks of storage (4-8°C) ($a_w$ ~ 0,83 @ 24.2°C)

Stability in Dry-Waffle Mix:




Half-Life (phase-one-decay): ~11-28 Days

**→ long stability within matrix**

# Conclusion

1) STEC-prevalence in ready-to-bake cookie dough seems to be low (underestimation due to sample volume possible)


3/4) at home baking procedures sufficient to inactivate STEC in dough at low contamination levels if dough is homogeneous and baked through

2) contamination in ready-to-bake cookie dough very low (<0,4 MPN / 100g)
& multiple contaminants possible

5) STEC survive cookie dough (> 6 month / > 4 weeks) and dough dry-mixes (>70 days)

# Outlook-What‘s next

- more surveillance of prevalence & contamination levels of STEC in flour and products thereof

  → strain characterization and more specific risk assessment

- research regarding inactivation rates in different kinds of dough

  → influence of factors like fat content, sugar content etc.

- effect of experimental procedures (i.e., spiking method, recovery medium)

  → more reliable & comparable results

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@ BfR:

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# Thank you for your attention

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# References


(BVL), B. f. V. u. L. (2019). Zoonosemonitoring 2018. Berichte zur Lebensmittelsicherheit.

Beuchat, L. R., E. Komitopoulou, H. Beckers, R. P. Betts, F. Bourdichon, S. Fanning, H. M. Joosten and B. H. Ter Kuile (2013). "Low-Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens." Journal of Food Protection 76(1): 150-172.

Boss, R. and J. Hummerjohann (2019). "Whole Genome Sequencing Characterization of Shiga Toxin-Producing Escherichia coli Isolated from Flour from Swiss Retail Markets." J Food Prot 82(8): 1398-1404.

CDC. (2016). "https://www.cdc.gov/ecoli/2016/o121-06-16/index.html." Retrieved 08.10.2019, 2019.

CDC. (2019). "https://www.cdc.gov/ecoli/2019/flour-05-19/index.html,." Retrieved 07.10.2019, 2019.

CDC (2021). https://www.cdc.gov/ecoli/2021/o121-07-21/details.html. Retrieved 02.12.2021, 2021.

Crowe, S. J., L. Bottichio, L. N. Shade, B. M. Whitney, N. Corral, B. Melius, K. D. Arends, D. Donovan, J. Stone, K. Allen, J. Rosner, J. Beal, L. Whitlock, A. Blackstock, J. Wetherington, L. A. Newberry, M. N. Schroeder, D. Wagner, E. Trees, S. Viazis, M. E. Wise and K. P. Neil (2017). "Shiga Toxin-Producing E. coli Infections Associated with Flour." N Engl J Med 377(21): 2036-2043.

Forghani, F., M. den Bakker, A. N. Futral and F. Diez-Gonzalez (2018). "Long-Term Survival and Thermal Death Kinetics of Enterohemorrhagic Escherichia coli Serogroups O26, O103, O111, and O157 in Wheat Flour." Appl Environ Microbiol 84(13).

Gieraltowski, L., C. Schwensohn, S. Meyer, D. Eikmeier, C. Medus, A. Sorenson, M. Forstner, A. Madad, J. Blankenship, P. Feng and I. Williams (2017). "Multistate Outbreak of Escherichia coli O157:H7 Infections Linked to Dough Mix - United States, 2016." Mmwr-Morbidity and Mortality Weekly Report 66(3): 88-89.

Gill, A., T. McMahon, F. Dussault and N. Petronella (2020). "Shiga toxin-producing Escherichia coli survives storage in wheat flour for two years." Food Microbiol 87: 103380.

Kindle, P., M. Nuesch-Inderbinen, N. Cernela and R. Stephan (2019). "Detection, Isolation, and Characterization of Shiga Toxin-Producing Escherichia coli in Flour." Journal of Food Protection 82(1): 164-167.

Knüppel, L. (2021). Considerations on STEC in ready-to-bake cookie dough:Heat tolerance and inactivation of Shiga toxin producing E. coli in flour-based products. Bachelor of Scrience, Freie Universität Berlin.

Made, D., A. C. Geuthner, R. Imming and A. Wicke (2017). "Detection and isolation of Shiga-Toxin producing Escherichia coli in flour in Germany between 2014 and 2017." Journal Fur Verbraucherschutz Und Lebensmittelsicherheit-Journal of Consumer Protection and Food Safety 12(3): 245-253.

Morton, V., J. M. Cheng, D. Sharma and A. Kearney (2017). "Notes from the Field: An Outbreak of Shiga Toxin-Producing Escherichia coli O121 Infections Associated with Flour - Canada, 2016-2017." MMWR Morb Mortal Wkly Rep 66(26): 705-706.

Neil, K. P., G. Biggerstaff, J. K. MacDonald, E. Trees, C. Medus, K. A. Musser, S. G. Stroika, D. Zink and M. J. Sotir (2012). "A novel vehicle for transmission of Escherichia coli O157:H7 to humans: multistate outbreak of E. coli O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough--United States, 2009." Clin Infect Dis 54(4): 511-518.

Schlager, S., C. Schlagenhaufen, S. Neubauer, K. Hauser, E. Edler, B. Springer and F. Allerberger (2018). Prevalence of Shigatoxin-Producing Escherichia coli in Different Types of Flour. ASM Microbe, Atlanta.

Sentker, M. (2021). Studies on E. coli strains including STEC in liquid dough. Bachelor of Science, Beuth University of Applied Sciences.