Health Canada Santé Canada

Canada

Baking flour as a vehicle for the transmission of Shiga toxin producing *Escherichia coli*

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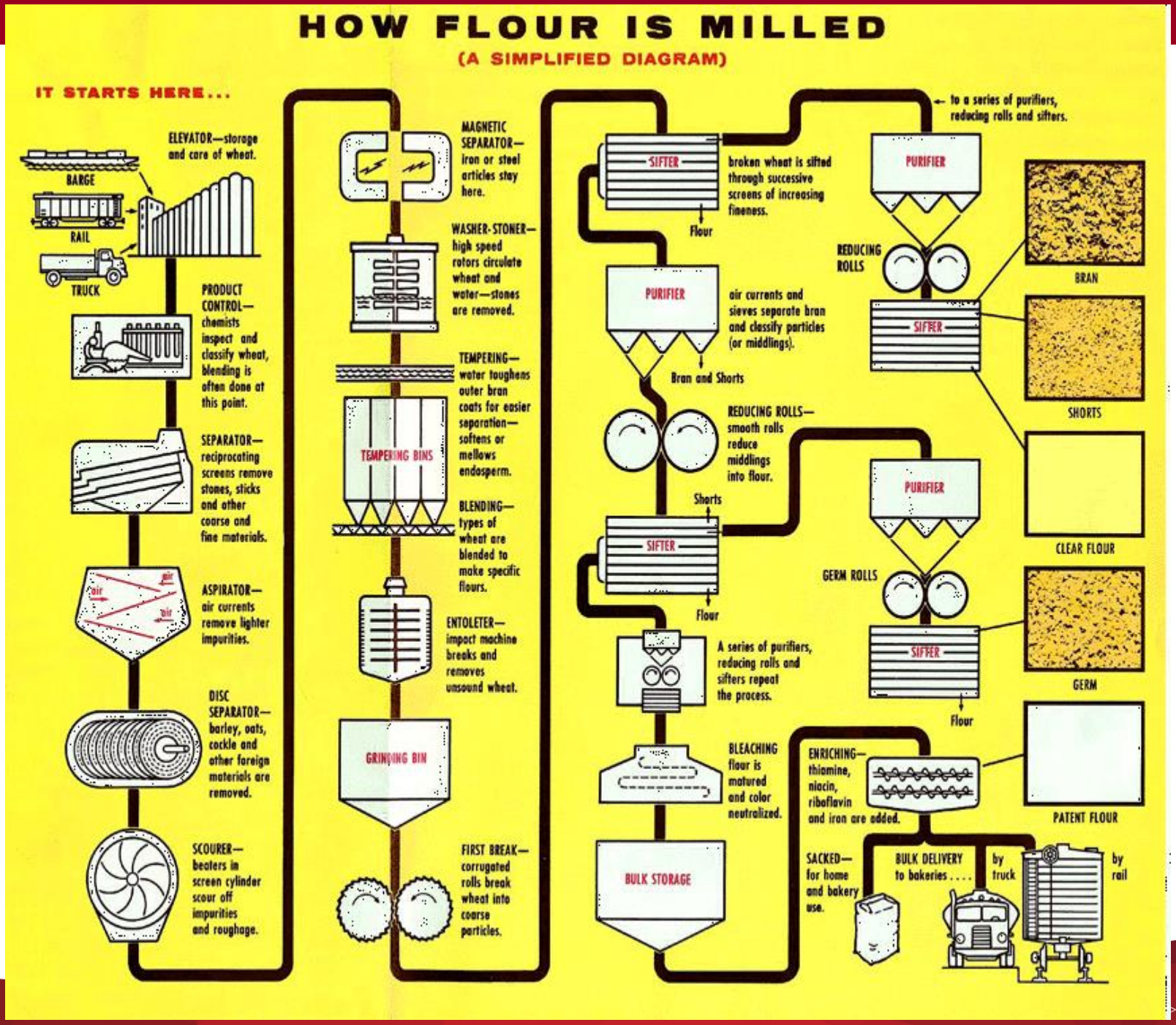
Grain Milling

Milling Economics – Canada

- The primary market for wheat flour is industrial bakers
- Less than 10% of flour is sold to consumers
- Industrial bakers require specific functional and rheological properties.
- Grain with different characteristics is milled and the flour mixed to create desired products


HOW FLOUR IS MILLED
(A SIMPLIFIED DIAGRAM)
IT STARTS HERE...
ELEVATOR—storage and care of wheat.
BARGE
RAIL
TRUCK
PRODUCT CONTROL—chemists inspect and classify wheat, blending is often done at this point.
SEPARATOR—reciprocating screens remove stones, sticks and other coarse and fine materials.
air
air
air
ASPIRATOR—air currents remove lighter impurities.
DISC SEPARATOR—barley, oats, cockle and other foreign materials are removed.
SCOURER—beaters in screen cylinder scour off impurities and roughage.
MAGNETIC SEPARATOR—iron or steel articles stay here.
WASHER-STONER—high speed rotors circulate wheat and water—stones are removed.
TEMPERING—water toughens outer bran coats for easier separation—softens or mellows endosperm.
TEMPERING BINS
BLENDING—types of wheat are blended to make specific flours.
ENTOLETER—impact machine breaks and removes unsound wheat.
GRINDING BIN
FIRST BREAK—corrugated rolls break wheat into coarse particles.
SIFTER
broken wheat is sifted through successive screens of increasing fineness.
Flour
PURIFIER
air currents and sieves separate bran and classify particles (or middlings).
Bran and Shorts
REDUCING ROLLS—smooth rolls reduce middlings into flour.
Shorts
SIFTER
Flour
A series of purifiers, reducing rolls and sifters repeat the process.
BLEACHING flour is matured and color neutralized.
BULK STORAGE
← to a series of purifiers, reducing rolls and sifters.
PURIFIER
REDUCING ROLLS
SIFTER
BRAN
SHORTS
CLEAR FLOUR
PURIFIER
GERM ROLLS
SIFTER
GERM
Flour
ENRICHING—thiamine, niacin, riboflavin and iron are added.
PATENT FLOUR
SACKED—for home and bakery use.
BULK DELIVERY to bakeries....
by truck
by rail

Bacteriology of Milling

Grain processing is non linear

- A lot of flour is not produced from a single load of grain
- Constant recirculation of grain particles and mixing
- Decontamination prior to packing likely futile

Heat treated flour

- Produced as a special product line
  - i.e. cookie dough ice cream
- Heating destroys functional characteristics

- No wet cleaning or sanitation.
  - Blowing/vacuuming of dust
  - Pest control – fumigation or heat

Bacteriology of Milling

Millers do not routinely test for bacteria

- Microbial growth controlled by low water activity <0.600
- Initial bacterial load on grain varies
  - Milling reduces total CFU/g by approx. 1 log
  - 3.5 to 4.5 log CFU/g is “normal”
- North America mean *E. coli* 0.82 log CFU/g

Milling operation is very dry.

- Moisture is very carefully controlled
- Two potential places for *E. coli* growth in the mill
  - Tempering bin
  - Animal Pests, e.g. rodents, beetles

Sabillón Galeas, 2014. http://digitalcommons.unl.edu/foodscidiss/49
Sperber et al. 2007. J. Food Prot. 70:1041
Eglezos 2010. J Food Prot. 73:1533
Berghofer et al. 2003. Int J Food Microbiol. 85:137

STEC in Flour

Outbreaks of STEC

Table 1. Outbreaks of STEC implicating wheat flour

Location	Dates	Cases	Serotypes
USA - Multistate	Dec 2015/Sept 2016	56	O121, O26
Canada - Multiprovince	Nov 2016/Apr 2017	30	O121
Canada - BC	Feb 2017/Sept 2017	6	O121
USA - Multistate	Dec 2018/May 2019	21	O26

Consumption of raw dough identified as a risk factor in two outbreaks

Crowe et al., 2017. N. Engl. J. Med. 377:2036.
BCCDC. 2017. http://www.bccdc.ca/about/news-stories/news-releases/2017/bccdc-advises-british-columbians-about-a-new-outbreak-of-e-coli-o121-associated-with-flour
Morton et al., 2017. Can. Commun. Dis. Rep. 43(7/8):154
CDC. 2019. https://www.cdc.gov/ecoli/2019/flour-05-19/index.html

STEC Prevalence in Milled Grains

Table 2. Prevalence of STEC in milled grains.

Country	n	Analytical unit	Grains	Prevalence *stx*	Prevalence STEC
Switzerland	93	25 g	wheat (52), wheat + other (19), spelt (18), rye (3) buckwheat (1)	10.8%	8.6%
Switzerland	70	5 x 10 g	wheat (21), spelt (14), rye (4), buckwheat (2), millet (2), chestnut (1), corn (1), durum wheat, (1) Emmer wheat (1), soy (1), mixed flour (22)	12.9%	11.4%
Germany	51	25 g	wheat, rye	29.4%	21.6%

Boss and Hummerjohann, 2019. J. Food Prot. 82(8):1398
Kindel et al., 2019. J. Food Prot. 82(1):164
Mäde et al. 2017. J. Consum. Prot. Food Saf. 12:245–253

Canada 2016/2017 Outbreak STEC O121:H19

Outbreak of STEC O121:H19

Outbreak Dates

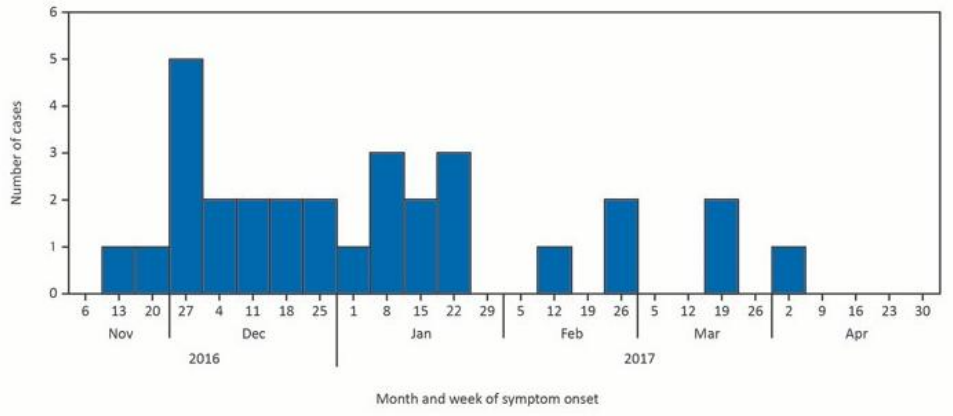
- Start: Nov 2016 End: Apr 2017
- Source identified in Mar 2017

Cases

- 30 confirmed in six provinces
- Age range 2-79 years (median 23.5 years)
- 8 hospitalisations / 1 hemolytic uremic syndrome

Outbreak strain of *E. coli*

- Serotype O121:H19
- Virulence genes
  - *stx2a*, *eae, hlyA*



Morton et al., 2017. Can. Commun. Dis. Rep. 43(7/8):154

Flour as the Source

Alberta Clinical Case

- STEC O121:H19
- Same PFGE as outbreak strain
- STEC O121:H19 isolated from open bag of flour

Canadian Food Inspection Agency

- Tested flour samples from implicated producer
- STEC O121 in flour milled on three sequential days in Oct 2016

Research Questions

What is the concentration of STEC O121 in the recalled flour?

- Exposure risk
- Choice of analytical sample size
- Pathogen infectivity

What is the composition of the microbiota of the samples?

- Understanding the contamination source
- Are there potential indicator organisms?

Characterisation of the pathogen

- Are there attributes which may have contributed to the outbreak?

Enumeration of Microbiota

STEC O121 enumeration by two approaches

- MPN enumeration of recalled product samples
- MPN statistics to estimate from qualitative data
  - Positive/negative results from outbreak investigation

Microbiota

- Total Aerobic Count
- MacConkey Agar (Gram negatives)
- Petrifilm *E. coli* and total Coliforms
- Composition of microbiota, genus level
- Identification of colonies from Total Aerobic plates
- Biochemical and Bruker Biotyper (MALDI-TOF)

STEC O121 in Flour

Table 3. Estimate of STEC O121 in wheat flour. Estimate from results of outbreak investigation testing. Estimate from MPN analysis of recall samples.

	MPN/100g	
Production Day	Estimate from Qualitative data	MPN Analysis
A	0.41	0.17
B	0.30	0.43
C	0.15	0.30

Gill et al. 2019. Food Micro. 82:474

Multiple STEC in Recalled Flour

Multiple STEC isolated

- O8:H28 (*stx1* -, *stx2a* +, *eae* -, *hlyA* +)
- O146:H21 (*stx1* -, *stx2b* +, *eae* -, *hlyA* +)

No disease cases associated with these serotypes in the outbreak period

- Lower potential to cause illness than STEC O121:H19?
- Lower infectivity than STEC O121:H19?
- Co-infection with STEC O121:H19?
- Were illnesses undiagnosed?

Gill et al. 2019. Food Micro. 82:474

Comparison of Recalled vs Retail Flour

No STEC in the 8 samples of Retail flour

- 5 x 100g analytical units for each sample
- Enrichment and PCR screen for *stx*

No evidence of more fecal bacteria in Recalled flour

Table 4. Enumeration of microbiota. Means are Log CFU/g. CL: 95% confidence limit. *Recalled significantly different than Retail, *t-test* (P <0.05)

Sample		ACC		Coliforms		*E. coli*	MAC	
	n	Mean	CI	Mean	CL	>10 cfu/g	Mean	CL
Recall A	6	4.9*	0.2	2.9	0.6	0	4.6*	0.2
Recall B	18	4.5*	0.3	3.0	0.1	1	4.5*	0.1
Recall C	6	4.5*	0.2	2.7	0.8	2	4.4*	0.2
Retail flour	24	3.9	0.2	2.7	0.1	0	3.7	0.2

ACC: total Aerobic Colony Count. Coliforms/ *E. coli*: 3M Petrifilm *E. coli*/Coliform. MAC: MacConkey agar

Gill et al. 2019. Food Micro. 82:474

Flour Analysis - 2 Years Storage

- No increase in water activity during storage
- Samples from two production days, A and B
- n=5 100 g analytical units form each day
- STEC O121 isolated
  - Same core genome Multilocus Sequence Type as outbreak isolates (max. 7 SNP's in 2513 genes)

Table 5. STEC O121:H19 in flour stored for 2 years

Production Day	n=5 100 g	MPN/100g
A	1/5	0.22
B	1/5	0.22

Delayed Lactose Phenotype

STEC O121:H19 strains from Canada and US flour outbreaks

- Do not utilise lactose in 24 h
- Lactose utilisation after 48 h
  or in 24 h when sub-cultured to a second lactose media

Table 6. Prevalence of delayed lactose phenotype in STEC

	β-Galactosidase Activity		
	Induced	**Delayed**	**No Activity**
O121:H19	12	11	1
O121:other H	7	1 (O121:NM)	-
Other O-types	36	-	1 (O145:H34)

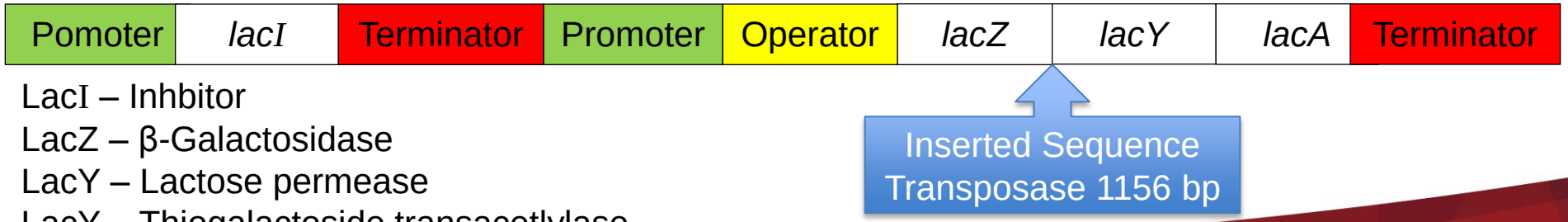
Delayed Lactose Phenotype

Genomic Analysis

- Long read scaffold (MinION)/Short read correction (MiSeq)
- Comparison of Lac operon in three O121:H19 strains

Lactose Phenotype	Strain ID	Isolate Origin	Comments
Induced (WT)	11-3925	Clinical	No Insert
Delayed	BMH-17-0004 19-9255	Flour Clinical	Inserted Sequence between *lacZ* and *lacY*
No Activity	1748	Clinical	Inserted Sequence between *lacZ* and *lacY* Silent T substitution in *lacI*

- Inserted sequence (Transposase 1156 bp)
  - Replaces 4 a.a. with 10 a.a. in end of LacZ
  - Increased Rho independent terminator sequences



LacI – Inhbitor
LacZ – β-Galactosidase
LacY – Lactose permease
LacY – Thiogalactoside transacetlylase

Conclusions

Are milled grains a new vehicle for STEC?

- Many unattributed STEC cases
- Outbreaks with wide geographic and temporal range
  - Better linking of cases
- Investigator awareness post 2016
- Milling is a traditional industry; no changes in processing

Are outbreaks caused by unusual contamination?

- *E. coli* is a normal part of flour microbiota
  - STEC are a subpopulation of *E. coli*
- No increase in fecal indicators in outbreak flour
- Surveys indicate STEC present in non-outbreak flour
- Illness associated with STEC at <1 MPN/100g

Conclusions

Need for robust sampling plans

- STEC in flour at <1 MPN/100g can cause outbreaks

Do not terminate analysis on first STEC isolation

- Multiple STEC can be present in a flour production lot

STEC O121:H19 can persist in flour up to 2 years

- Is this exceptional among STEC?
- Are other low moisture foods STEC vehicles?

Pathogen levels can be estimated from qualitative data

- Treat positive/negative results as MPN tubes
- Can potentially guide sampling plans

Acknowledgements

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