#### **Request:**

What are the pathogens of concern in raw milk extra hard cheese varieties such as Parmesan, Grana Padano, and similar types?

### **Response:**

#### Overview

The main pathogens of concern in natural, raw-milk cheeses are *L. monocytogenes*, Shiga toxinproducing *Campylobacter* spp., *E. coli* (STEC), *Salmonella* spp., and *Staphylococcus aureus* (Donnelly, 2018, Possas et al., 2021), although the risks of these pathogens in raw-milk, extra-hard cheeses have been classified as negligible, very low, or low (Food Standards Australia New Zealand, 2009). This literature review attempted to identify newer data suggesting greater or new pathogenic hazards in raw-milk, extra-hard cheeses.

The search focused mainly on publications since 2009, when "Microbiological Risk Assessment of Raw Milk Cheese" was published (Food Standards Australia New Zealand, 2009). The terms used in searches initially include the following: "extra hard" cheese varieties: Parmesan, Grana Padano, Pecorino, Asiago, and Sbrinz. These cheeses typically have a moisture level of <36% and are made using internal bacterial ripening. Most are made with a relatively high curd-cooking temperature (>45°C) and long storage/maturation times (8-24 months) (Food Standards Australia New Zealand, 2009).

Other cheeses such as Cotija and Manchego may range from semi-hard to extra hard and were also considered in searches (Ibarra-Sánchez et al., 2017). There are some differences between how hard and extra-hard cheeses are distinguished around the globe (Gobbetti and Di Cagno, 2017), and many studies do not report moisture content of cheeses in individual studies. For this reason, some information on hard cheeses is also included at the end of this summary.

## Outbreaks of foodborne disease associated with extra-hard cheeses

Foodborne disease outbreaks associated with extra-hard cheese have only rarely, if ever, been reported in recent years.

- A list of foodborne disease outbreaks associated with cheese from 1980 to 2013 did not include any outbreaks specifically associated with raw-milk, extra-hard cheeses (Fox et al., 2017).
- An outbreak of *Salmonella* Newport attributed to Cotija cheese occurred in the U.S. during 2006-2007 (Austin et al., 2008); however, Cotija cheeses may range in hardness from semi-hard to extra hard, and the moisture level of the cheese in this outbreak was not reported.

## Identification and persistence of pathogens in extra-hard raw milk cheeses

Pathogens have been identified in raw milk extra-hard cheeses.

- Salmonella enterica has been found in raw sheep's milk pecorino cheese in Italy (Ercoli et al., 2015).
- Cotija cheese in the U.S. was recalled in 2020 because of possible contamination with STEC.
- *S. aureus* has been found in Cotija cheese in Mexico (Adame-Gomez 2018, Toribio-Jimenez 2014) and in Italian raw milk pecorino cheese (Normanno et al., 2007).

Several studies have tested to see how long pathogens could survive in raw milk extra-hard cheeses:

- *L. monocytogenes* could survive at a stable level in pecorino cheese for ~1500 hours (~60 days) at 18°C, but then decreased quickly to 0 log CFU by 2500 hours (104 days) (Centorotola et al., 2020).
- *E. coli* O157 survived 90 days of aging, but not at 120 days of aging in raw sheep's milk pecorino cheese. (Cardamone et al., 2018).
- Another study from the same group, however, found that *E. coli*, *L. monocytogenes*, *Salmonella* spp., and *S. aureus* disappeared completely after 60 days of ripening in raw milk pecorino cheese (Cardamone et al., 2020).

Other pathogens of potential concern have been found in extra-hard cheeses:

- *Mycobacterium avium* subsp. *paratuberculosis,* which may be link to Crohn's disease, survived until the second or third month of ripening in Parmigiano Reggiano and Grana Padano cheeses when spiked into raw bovine milk (Cammi et al., 2019).
- *Mycobacterium bovis* (inoculated into pasteurized milk) has been found to decline slowly and still be present after 60 days of maturation in parmesan-type cheese (Starikoff et al., 2016).
- Brucella spp. has been found in hard cheeses in Europe (Jansen et al., 2019); an outbreak of brucellosis occurred in England in 1965 which traced back to pecorino cheese made from unpasteurized sheep's milk cheese which had ripened for more than 90 days (Galbraith et al., 1969). B. abortus (inoculated into pasteurized milk) was able to survive 20 days during maturation of a parmesan-type cheese (Starikoff et al., 2016).

## Hard Cheeses

In contrast to extra-hard cheese, raw milk hard cheeses have been associated with a number of outbreaks, including the following (not an exhaustive list):

- A 2006 outbreak of *Salmonella* spp. in the Netherlands was associated with raw-milk hard cheese (Van Duynhoven et al., 2009).
- A 2017 review lists outbreaks of pathogenic *E. coli* in aged gouda and other hard cheese, numerous *Salmonella* outbreaks in cheddar cheese, and *S. aureus* outbreaks in Cheddar cheese (*Fox et al., 2017*).
- A 2018 review states that no listeriosis outbreaks have been linked to hard cheeses, (Gérard et al., 2018).

Pathogens are more commonly found in hard cheeses than in extra-hard cheeses:

- A 2018 review cites a number of studies in which *L. monocytogenes* has been found in hard cheeses (Gérard et al., 2018)
- A 2016-2018 FDA survey of 1606 raw milk cheeses did not appear to look at any "extra hard cheeses" (but may not have distinguished between hard and extra hard cheese, as they defined hard cheeses as no more than 39% moisture). Pathogens were found in some raw-milk hard cheeses, including *Salmonella* spp. in 1/521 samples, *L. monocytogenes* in 2/521 samples, pathogenic STEC in 1/521 samples (FDA, 2016).

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