September 12, 2022 Cheese Brine Composition and Microbiology

Request: We are interested in learning more about the composition and microflora (especially yeasts) of brines used with Gouda-style cheeses.

Response:

Gouda cheese brine composition, etc.

In Gouda cheeses:

- An older paper (1976) reported a brine for Gouda cheese was typically 17% common salt with a pH of 4.6, with a salting temperature of 13°C (Berg et al., 1976).
    - This paper also discusses yeast in the brine, which is more of a problem with a "weak" or lower salt brine. Aeration of the brine with a circulating device (especially if small air bubbles are produced) stimulated yeast growth.
    - This paper also described a survey of a number of Gouda brine samples collected in practice, finding that the salt percentages ranged from 11.2 to 20.2, with pH values ranging from 4.15 to 5.01 (Berg et al., 1976).
- Another paper reported using a brine of 180 to 200 g NaCl per kg of brine, pH 5.15 to 5.30, but this was a laboratory-scale brine (Domagała et al., 2022)
- "The brine solutions at BK and HH (two artisan producers of Gouda cheese) differed in the concentration of salt (expressed as degrees Baumé, degrees Bé, often used as a measure for the salinity of the brining solution), 20 degrees Bé and 18 degrees Bé at BK and HH, respectively" (Van Hoorde et al., 2010).
- A dairy factory cooperative (apparently in the Netherlands) analyzed the brine from numerous cheese plants in the 1970s. The pH of most brines was between 4.41 and 4.8, as shown in Table 1 of the Hansen reference (Hansen, 1979).
- Another study in the same paper which looked at cheese brines in Austria found that pH of most brines (55.8%) ranged from 5.10 to 5.30, with 35% of brines at pH levels above 5.3. The temperature of most of the brines (58%) was between 10 and 16°C, while 37% were at higher temperatures (as high as 26°C) (Hansen, 1979).

In other cheese types:

- One paper reported that Grana cheese brine could be regenerated by raising it to a high pH with NaOH to precipitate calcium and phosphorus salts. To restore the original brine's pH, HCL was used (Bertelli et al., 1997).

Types of microflora present:

The brine from Danish dairies producing Danbo cheese (a surface ripened, semi-hard cheese, submerged in brine for up to 24 hours before ripening) was studied (Haastrup et al., 2018).

- pH of brines varied from 5.1 to 5.6
- Lactate varied from 4.1 to 10.8 g/L
- Bacteria isolated from the brines (which differed between dairies) at levels >6 log CFU/mL were *Tetragenococcus muriaticus* and *Psychrobacter celer*. Others present at levels >4.5 log CFU/mL

included *Lactococcus lactis*, *Staphylococcus equorum*, *Staphylococcus hominis*, *Chromohalobacter beijerinckii*, *Chromohalobacter japonicus* and *Microbacterium maritypicum*.
- *Debaryomyces hansenii* was the only yeast found at levels greater than 3.5 log CFU/mL.
- "It seems clear that the brine not only contributes to the structural and organoleptic properties
- of the cheeses, but also serves as an important source for surface inoculation of the un-ripened cheeses with a wide range of halotolerant and/or halophilic microorganisms". These organisms appear to be introduced through the sea salt used in the brine (Haastrup et al., 2018).

Another recent study looked at the bacterial and physiochemical diversity within Italian cheese brines used for a variety of different types of cheeses (soft, semi-hard, and hard) (Marino et al., 2017).

- Brine pH ranged from 4.64 to 5.67, with highest pH for the hard cheeses.
- Temperatures of brines ranged from 8 to 16°C, with the highest temperatures for hard cheeses.
- The most abundant families were Lactobacillaceae, Moraxellaceae, Halomonadaceae, Streptococcaceae, Micrococcaceae, Pseudomonadaceae and Staphylococcaceae.

Another recent paper used culture independent methods to characterize the microbiota of cheese brines from an artisan and a large commercial cheese maker in Belgium (Vermote et al., 2018). "*Debaryomyces* (family Debaryomycetaceae) was the only yeast genus found with shotgun metagenomic sequencing as well as artisan cheese brine, whereas in the large-scale cheese brine, also the polyphyletic genus *Candida* was found with amplicon sequencing. Indeed, metagenomic recruitment plotting showed the presence of the yeast species *D. hansenii* in the large-scale cheese brine, as well as another phylogenetically related species in both brines".

One older paper looked at yeasts associated with cheddar and gouda production (not just brine) at a single factory (Viljoen and Greyling, 1995).

- The authors concluded "The most prevalent isolates belonged to the genera *Debaryomyces* and *Candida*.
- Other genera encountered were *Cryptococcus, Rhodotorula, Yarrowia, Pichia, Trichosporon, Torulaspora, Issatchenkia, Saccharomyces* and *Zygosaccharomyces*.
- "Characterization of the predominant yeast isolates indicated that the cheese brine was responsible for the largest variety and number of yeast isolates yielding a total of 64 yeast strains belonging to nine different genera."

An older review discussed the many types of yeasts that are isolated from cheese brines (Seiler and Busse, 1990)

Effect of brine components on yeasts:

- *Debaryomyces hansenii* strains isolated from Danish cheese brines can inhibit germination and growth of contaminating molds (Huang et al., 2021).

- An earlier paper found that *D. hansenii* isolates from cheese produced mycocins that are effective against *Candidia albicans* and *Candidia tropicalis* over a range of temperatures and pHs (Banjara et al., 2016).
- A recent paper examined the effect of different growth conditions on the growth and survival of yeasts isolated from Danish cheese brines (Zhang et al., 2020).
    - *D. hansenii* (contrary to other reports) grew best at 4% NaCl.
    - Under brine-mimicking conditions (high salt), only one strain of *D. hansenii* tested was able to grow at 25°C, while nearly all yeast strains could grow at 16°C. At low salt conditions (4% salt), all strains grew at both temperatures, with better growth at 25°C.
- An earlier paper (which didn't look at brines) found that the apparent antifungal activity of certain dairy isolates of lactic acid bacteria (which was only apparent at pH levels below 5) was due to acetic acid in the media (Cabo et al., 2002). Acetic acid has a pKa of 4.75. The discussion of the paper reviews the relatively low effect of lactic acid alone (pKa of 3.8) against yeast and molds.

Effects of organic acids in brines on other microorganisms:

- In a brined Greek cheese, the addition of 1% of tartaric, malic, ascorbic, fumaric, lactic, acetic, and succinic acids was assessed for its affects against the growth of lactic acid bacteria, yeasts/molds, and *L. monocytogenes*. Citric acid had better antifungal activity than tartaric acid (Tavsanli et al., 2019).

Other papers of possible interest:

- A 2015 paper demonstrated a significant reduction in microorganisms (including yeasts) in cheese brine by application of gaseous ozone (Marino et al., 2015).
- One recent study screened L. plantarum strains for antifungal activity against brine yeast contaminants for potential biocontrol options (Kavkova et al., 2022).

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