

Request: We are interested in some information on nisin, particularly related to dairy applications.

1. When in the growth cycle nisin is produced?
2. Do the presence of particular nutrients increase / decrease nisin production?
3. What levels of nisin are inhibitory to what species of bacteria?

Response:

Question 1:

- From a recent review (Khelissa et al., 2021): “For a long time, it was believed that nisin is synthesized during the stationary phase when nutrients are exhausted. However, it has been reported that in batch fermentation, the nisin production follows a primary metabolite kinetic (Guerra et al. 2007): a production during the exponential growth phase and a full stop when the bacteria enter the stationary phase. It was observed that nisin was detected in the growth medium during the exponential phase and its production rate peaked at the end of this phase which confirms that the synthesis of this peptide follows a primary metabolite kinetic (Chinachoti et al. 1998; Zhu 2017).
- Nisin expressed *in situ* by a costarter culture during the making of a hard Graviera cheese was investigated in Noutsopoulos (2017). Nisin was expressed to the highest level on Day 1 of fermentation (Figure 2), with “increasing nis A expression by the NisA+ costarter strain M78 during the exponential phase of microbial growth”.
- Another paper from the Samelis research group also looked at nisin expression in a co-culture of the *L. lactis* M78 (NisA + strain) with *Streptococcus thermophilus* in milk and looked how *S. thermophilus* inoculation level affected M78 growth and nisin A expression and activity vs. *L. monocytogenes*. A fairly low inoculation level of the *S. thermophilus* strain (5-log) together with a moderate level of the M78 NisA+ starter (6 log inoculation) was best to produce sufficient nisin A to provide good activity in milk (Samelis and Kakouri, 2020).
- Commercial production of nisin in a *L. lactis* monoculture in milk is usually optimized at pH 6.0 and 30°C. Fed batch systems are generally better than using high substrate concentrations in the media, and lactate accumulation can also adversely affect nisin production in *L. lactis*. Under such conditions, the highest nisin activity is generally found after 8-10 hours of fermentation. Nisin activity decreases dramatically after that, probably due to proteolytic degradation or adsorption of the nisin onto producer cells (Özel et al., 2018).

Question 2:

- Factors in culture media that influence bacteriocin production in LAB have been reviewed in several recent papers (Abbasiliasi et al., 2017; Khelissa et al., 2021; Peng et al., 2022):
 - A carbon source is essential, with glucose promoting bacteriocin production.
 - A nitrogen source is also essential, with yeast extract preferable to peptone or meat extract for bacteriocin production. Yeast extract may have more absorbable amino acids and short peptides (as well as more growth factors, minerals, and vitamins).
 - Potassium ion at 2 g/L has been found to promote bacteriocin production in MRS medium but has a negative effect in M17 medium.
 - Sodium decreases bacteriocin production in *L. lactis* subsp. *lactis* and other LAB.
 - Manganese is frequently reported to promote bacteriocin production (0.01 to 0.2 g/L).
 - Magnesium did not appear to stimulate or reduce bacteriocin production, while no conclusions can be drawn from other metal ions.

- An initial pH of 6-7 and temperature of 30-37°C is optimal for bacteriocin production in LAB. Oxygen is reported to stimulate the production of nisin.
- The discussion (Noutsopoulos et al., 2017) reviews factors that reduce nisin production by *L. lactis* during cofermentation hard cheese making (deprivation of sugars, amino acids, and other essential nutrients; the possibility of other bacteria excreting proteases, effects of temperature, pH, salt concentration and water activity, etc.
- A recent book chapter by Singh discusses nutrients and growth conditions for optimizing nisin production in media and in commercial applications (Singh, 2019).
- Another paper reviews other work which examined the effects of various factors on nisin production (Garcia-Parra et al., 2011). The paper itself tests various factors on nisin production in a new *L. lactis* strain and found various factors that improved production.

Question 3:

Bacillus cereus

- The MIC for nisin against *B. cereus* in milk at 21°C was 32 IU/mL.

Clostridium butyricum

- “Nisin, at concentrations of 2.50–6.24 µg/g, was shown to be extremely effective in preventing the growth of relevant *Clostridium* spp. inoculated in processed cheese and cheese spreads (with or without ham) incubated at 37°C. This effectiveness was also reported by Plocková et al. (1996) who found that similar nisin levels inhibited *Clostridium* and *Bacillus* in processed cheese during a 3-month storage period”.
- MICs for nisin in *C. tyrobutyricum*, *C. butyricum*, and other dairy-related clostridia ranged from 0.1 to 6.25 µg/mL for vegetative cells and 0.20 to 25 µg/mL for spores (Ávila et al., 2014).

Clostridium perfringens

- Nisin (MIC values 0.78 to 12.5 µg/ml) was also effective against vegetative cells and spores of *C. perfringens* in media and milk after 7 days at 37°C under anaerobic conditions (Garde et al., 2014). The paper also discusses a number of other papers that determined MIC values for nisin against *C. perfringens* in milk or media.
- Against *C. perfringens* vegetative cells, nisin was reported to lengthen the lag phase (Garde et al., 2014).

Listeria

- The MIC for nisin against *L. monocytogenes* in milk at 21°C was 16 IU/mL; at 30°C, it was 48 IU/mL.
- Against *L. monocytogenes* in cuajada cheese, nisin initially reduced growth of the pathogen (2.74 log CFU/g lower counts than control cheese after 3 days at 10°C); however, the extension of lag phase was followed by growth from days 3-9 similar to those found in the control cheese without nisin (Arques et al., 2008)

Staphs

- The MIC of nisin against various *S. aureus* strains was reviewed and ranged between 6.4 and 12.8 µg/mL.

- One study added nisin to a raw-milk Mexican cheese contaminated with *S. aureus*: “Addition of nisin during cheese production reduced ($P \leq 0.05$) *S. aureus* counts compared to a nisin-free control; at 24 h, nisin lowered *S. aureus* counts 2.3 log cycles in the 500 IU/kg nisin treatment and 1.9 log cycles in the 625 IU/kg treatment”. (Alvarez Badel et al., 2022)
- Nisin alone resulted in a significantly lower levels (~ 1 -2 log) of *S. aureus* in inoculated pasteurized milk after 24 hours of storage at 25 or 4°C (Li et al., 2022).
- Against *S. aureus* in milk, nisin was most effective during lag phase; log phase was not affected (Felicio et al., 2015). Similarly, Yehia (2022) and Jensen (2020) found that MRSA in culture was most sensitive early in the growth curve, and the loss in sensitivity after 4 hours of nisin exposure was not due to stable mutations (Yehia et al., 2022) (Jensen et al., 2020).
- In cheese curd and whey, nisin (which partitioned into both phases) was most effective in reducing high initial *S. aureus* counts in highly contaminated milk (Felicio et al., 2015).

References

- Abbasiliasi, S., J. S. Tan, T. A. Tengku Ibrahim, F. Bashokouh, N. R. Ramakrishnan, S. Mustafa, and A. B. Ariff. 2017. Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: A review. *RSC Advances* 7:29395-29420.
- Alvarez Badel, B., M. A. Doria Espitia, V. Hodeg Peña, M. M. Simanca Sotelo, Y. Pastrana Puche, and C. D. D. Paula. 2022. Efecto de la nisina en la inhibición del crecimiento de *staphylococcus aureus* y en las propiedades sensoriales del queso costeño. *Revista mexicana de ciencias pecuarias* 13:272-286.
- Arques, J. L., E. Rodriguez, M. Nunez, and M. Medina. 2008. Antimicrobial activity of nisin, reuterin, and the lactoperoxidase system on *listeria monocytogenes* and *staphylococcus aureus* in cuajada, a semisolid dairy product manufactured in Spain. *J. Dairy Sci.* 91:70-75.
- Ávila, M., N. Gómez-Torres, M. Hernández, and S. Garde. 2014. Inhibitory activity of reuterin, nisin, lysozyme and nitrite against vegetative cells and spores of dairy-related *clostridium* species. *Int. J. Food Microbiol.* 172:70-75.
- Felicio, B. A., M. S. Pinto, F. S. Oliveira, M. W. Lempk, A. C. S. Pires, and C. A. Lelis. 2015. Effects of nisin on *staphylococcus aureus* count and physicochemical properties of Minas Frescal cheese. *J. Dairy Sci.* 98:4364-4369.
- García-Parra, M. D., B. E. García-Ahmendarez, L. Guevara-Olvera, R. G. Guevara-Gonzalez, A. Rodriguez, B. Martinez, J. Dominguez-Dominguez, and C. Regalado. 2011. Effect of sub-inhibitory amounts of nisin and mineral salts on nisin production by *Lactococcus lactis* uq2 in skim milk. *Food and Bioprocess Technology* 4:646-654.
- Garde, S., N. Gomez-Torres, M. Hernandez, and M. Avila. 2014. Susceptibility of *clostridium perfringens* to antimicrobials produced by lactic acid bacteria: Reuterin and nisin. *Food Control* 44:22-25.
- Jensen, C., H. Li, M. Vestergaard, A. Dalsgaard, D. Frees, and J. J. Leisner. 2020. Nisin damages the septal membrane and triggers DNA condensation in methicillin-resistant *staphylococcus aureus*. *Front. Microbiol.* 11.
- Khelissa, S., N. E. Chihib, and A. Gharsallaoui. 2021. Conditions of nisin production by *Lactococcus lactis* subsp. *Lactis* and its main uses as a food preservative. *Arch. Microbiol.* 203:465-480.
- Li, Q. X., S. N. Yu, J. Z. Han, J. L. Wu, L. J. You, X. D. Shi, and S. Y. Wang. 2022. Synergistic antibacterial activity and mechanism of action of nisin/carvacrol combination against *staphylococcus aureus* and their application in the infecting pasteurized milk. *Food Chem.* 380.

- Noutsopoulos, D., A. Kakouri, E. Kartezini, D. Pappas, E. Hatziloukas, and J. Samelis. 2017. Growth, nisa gene expression, and in situ activity of novel lactococcus lactis subsp cremoris costarter culture in commercial hard cheese production. J. Food Prot. 80:2137-2146.
- Özel, B., Ö. Şimşek, M. Akçelik, and P. E. J. Saris. 2018. Innovative approaches to nisin production. Applied Microbiology and Biotechnology 102:6299-6307.
- Peng, Z., T. Xiong, T. Huang, X. Y. Xu, P. R. Fan, B. L. Qiao, and M. Y. Xie. 2022. Factors affecting production and effectiveness, performance improvement and mechanisms of action of bacteriocins as food preservative. Critical Reviews in Food Science and Nutrition. doi:10.1080/10408398.2022.2100874.
- Samelis, J. and A. Kakouri. 2020. Cell growth density and nisin a activity of the indigenous lactococcus lactis subsp. Cremoris m78 costarter depend strongly on inoculation levels of a commercial Streptococcus thermophilus starter in milk: Practical aspects for traditional greek cheese processors. J. Food Prot. 83:542-551.
- Singh, S. 2019. Nisin production with aspects on its practical quantification. Microbial Interventions in Agriculture and Environment, Vol 1: Research Trends, Priorities and Prospects:545-596.
- Yehia, H. M., A. F. Alkhuriji, I. Savvaids, and A. H. Al-Masoud. 2022. Bactericidal effect of nisin and reuterin on methicillin-resistant staphylococcus aureus (mrsa) and s. Aureus atcc 25937. Food Science and Technology 42.

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