Evolving dairy: The A2 shift and its industry impact

Davor Daniloski [1, 2, 3]

[1] *The University of Queensland, School of Chemical Engineering, Queensland 4072, Australia*

[2] *Victoria University, Advanced Food Systems Research Unit, Institute for Sustainable Industries and Liveable Cities, Melbourne, Victoria 8001, Australia*

[3] *Teagasc Food Research Centre, Food Chemistry and Technology Department, Moorepark, Fermoy, P61 C996 Cork, Ireland*

Summary

Over the last century, the genetic selection in dairy cattle has significantly evolved, nowadays, the emphasis is on improving milk production, composition, and yield. Among these developments, selecting specific casein genotypes has emerged as a key factor influencing dairy processing and product quality. This factsheet explores cutting-edge and latest research on conventional and A2/A2 milk, particularly focusing on how β-casein variants affect dairy processing traits. The A2/A2 milk, with its distinct β-casein structure, presents notable processing challenges, such as reduced heat stability and less effective acid gelation and rennet coagulation. Understanding these differences is crucial for dairy producers to optimise product quality and address consumer health considerations.

1. Introduction

Bovine milk is an excellent source of nutrients, containing relatively high levels of proteins, lipids, carbohydrates, and minor components, such as minerals and vitamins, making it widely used in human nutrition (McSweeney & Fox, 2013). Milk protein can be mainly divided into two fractions, caseins, and whey proteins, present at a ratio of approximately 80:20 in mature bovine milk. The casein group of proteins can be further classified into four different types, namely αs_1-, αs_2-, β-, and κ-caseins (Chandrapala & Huppertz, 2024). Approximately 40 % of the total casein and one-third of the total protein content in bovine milk is β-casein (Atamer et al., 2017).

Bovine β-casein and its phenotypes have gained significant recognition within the dairy industry, as they have been related to affecting the casein micelle structure (Daniloski, Markoska, McCarthy, & Vasiljevic, 2023), techno-functionality of milk and dairy products (Daniloski, McCarthy, Huppertz, & Vasiljevic, 2022), and also have a potential impact on human health, including conditions such as digestive discomfort, diabetes mellitus, cardiovascular diseases, neurological disorders, and pulmonary inflammation (De Noni et al., 2009). While several β-casein variants have been identified, the most common are β-caseins A1 and A2 (Aschaffenburg, 1963). The defining feature of these two types of β-casein is the inclusion of either histidine in β-casein A1 or proline in β-casein A2 at position 67 in their polypeptide chains (Aschaffenburg, 1968). Namely, bovine milk comprised of the homozygous β-casein A2 is termed A2/A2 milk, whilst a mixture of both β-caseins A1 and A2 leads to the production of conventional or A1/A2 milk (Daniloski, 2024a).

The objective of this document is to provide the reader with sufficient references to gain an understanding of the complexity of β-caseins A1 and A2 as encountered in the manufacture of dairy products. However, the content of this document does by no means provide a legal opinion on the interpretation of the current international dairy standards. This document suggests that β-casein A2 is the dominant variant in casein micelles associated with impaired protein structure, reduced heat stability, and poor coagulation, negatively affecting the production of fermented dairy products, such as yogurt and cheese (Juan et al., 2024). The complexity of β-casein structure, along with various milk properties and processing methods, makes it challenging to attribute differences between conventional and A2/A2 milk solely to β-casein variants. Additionally, factors such as cow genetics, protein content, farming practices, and environmental conditions further influence these variations (Bijl, Holland, & Boland, 2020; Day, Williams, Otter, & Augustin, 2015). The current factsheet offers essential insights into how different β-casein variants affect dairy structures and the performance of dairy products, providing a clearer understanding of their functional differences.

2. The variants of β-casein and casein micelle

Casein micelles form through a complex interplay of primarily non-covalent interactions, with some covalent bonds and ionic interactions involving calcium phosphate nanoclusters (Lucey & Horne, 2018). The exact nature of these interactions remains debated: some emphasise hydrophobic interactions (Horne, 2020), while others prioritise backbone interactions (Carver & Holt, 2019). De Kruif, Huppertz, Urban, and Petukhov (2012) highlighted that casein interactions involve hydrophobic and hydrogen bonding, electrostatic forces, and van der Waals attractions. Various models have been proposed to explain the casein micelle assembly and structure, including the sub-micelle model (Slattery & Evard, 1973), dual-binding model (Horne, 1998), nano-cluster model (Holt, 1992), water channel model (Dalgleish, 2011), and network model (Huppertz et al., 2017). Despite extensive research, consensus on the exact interactions and structure of casein micelles remains elusive due to the complexity of visualising their structure (Carver & Holt, 2024). Understanding the intricate interplay of forces that govern casein micelle formation provides a foundation for exploring the role of specific casein proteins within this structure, particularly β-casein, which is essential in determining the micelle's functionality and behaviour in milk.

Bovine β-casein, the most hydrophobic of the caseins, plays a pivotal role in casein micelle formation, essential for the structure and functionality of milk. With a flexible, open conformation and a molecular mass of about 24 kDa after phosphorylation, β-casein's unique structure, particularly the phosphorylation of five key residues, drives its strong amphipathic nature, enabling vital interactions with calcium phosphate nanoclusters and other caseins (Atamer et al., 2017). The molecule's hydrophobic C-terminal and polar, negatively charged N-terminal allow it to self-assemble into β-casein micelles, forming a structure with a hydrophobic core and hydrophilic surface. This micellization process is primarily driven by hydrophobic interactions, following a stepwise model where dimer forms sequentially add to a growing micelle (De Kruif & Grinberg, 2002).

Recent research by Markoska (2023) highlights critical structural differences between β-caseins A1 and A2 at pH levels crucial for native milk conditions (pH 6.7) and milk gelation (pH 4.6). Using advanced techniques, such as microscopy, Fourier Transform Infrared and Raman

spectroscopy, but also ^{13}C Nuclear Magnetic Resonance, the study finds that β-casein A2 with its high presence of polyproline II motifs forms an open, porous network. In contrast, β-casein A1 shows intensified aggregation, which results in dense protein structures dominated by β-sheets (Markoska, 2023). The polyproline II motifs in β-casein A2 favour being conserved, leading to open and extended supramolecular structures (Thorn, Ecroyd, Carver, & Holt, 2015) and hence a larger β-casein micelle size. Whilst β-casein A1 forms smaller individual micelles in isolation, it also tends to result in smaller casein micelle sizes in milk compared to β-casein A2 (Day et al., 2015). Complementing this, Gustavsson et al. (2014) and Daniloski, McCarthy, Markoska, Auldist, and Vasiljevic (2022a) found that smaller casein micelles are linked to higher total and ionic calcium levels, with conventional milk showing greater calcium content and smaller micelles than A2/A2 milk. The main reason behind this difference is still not fully understood, but the aforementioned properties of conventional milk can have a positive impact on heat stability, gelation, and coagulation milk behaviour (Dumpler, Huppertz, & Kulozik, 2020).

3. How does β-casein impact the milk heat treatment and stability?

Heat treatment is widely used in the dairy industry to reduce bacterial and enzymatic activity, ensuring milk safety, and extending shelf life (Anema, 2021). However, it can also lead to gelling, coagulation, thickening, and fouling, making it crucial to understand milk's heat stability (Dumpler et al., 2020). During heat treatment, whey proteins like β-lactoglobulin denature and interact with casein micelles or form complexes in the serum phase, depending on the pH (Fox & Morrissey, 1977).

While extensive research has focused on κ-casein's effects on heat stability (Choi & Ng-Kwai-Hang, 2002; Fox & Hearn, 1978; Robitaille, 1995; Robitaille & Ayers, 1995), the impact of β-casein variants is less understood. At high temperatures, β-casein can act as a molecular chaperone, preventing aggregation of whey proteins (Kehoe & Foegeding, 2011). Daniloski, McCarthy, and Vasiljevic (2022b) found that β-casein A2 in A2/A2 milk possesses stronger chaperone activity, reducing heat-induced aggregation more effectively than β-casein A1 in conventional milk. This is reflected in heat coagulation times where A2/A2 milk exhibits lower heat stability compared to conventional milk likely due to its larger casein micelles with lower κ-casein content (Daniloski, Hailu, Brodkorb, Vasiljevic, & McCarthy, 2024b; Gai, Uniacke-Lowe, O'Regan, Goulding, & Kelly, 2023). This instability during heating can lead to undesirable changes in texture, consistency, and shelf life. Additionally, the higher amount of β-lactoglobulin/κ-casein complexes in A2/A2 milk may further affect the milk's ability to form stable gels or emulsions, greater aggregates, impacting the overall quality of the final product (Fox & Morrissey, 1977).

4. Fermented A2/A2 dairy products

The differences in gel strength, curd formation, and water-holding capacity among various milk types have profound implications for yogurt and cheese production. Research has shown that genetic variants of β-casein play a crucial role in determining these functional properties, thereby influencing the texture, firmness, and sensory characteristics of fermented dairy products (Juan et al., 2024).

Acid gels and yogurts made from conventional milk demonstrate superior gel strength, cohesiveness, and water-holding capacity compared to yogurt made from A2/A2 milk. The storage modulus of conventional yogurt is consistently higher, indicating a more robust gel matrix that resists deformation. This increased gel strength translates into higher firmness and consistency, making conventional yogurt less prone to syneresis, resulting in a smoother and denser texture. The microstructural analysis further supports these findings, revealing that A2/A2 yogurt is more porous and softer (Daniloski, McCarthy, Gazi, & Vasiljevic, 2022c; Daniloski et al., 2024c; Nguyen, Schwendel, Harland, & Day, 2018; Poulsen & Larsen, 2021).

Similar trends are observed in cheese production. Milk containing β-casein A2/A2 requires a longer rennet coagulation time and forms softer curds with lower firmness (Daniloski et al., 2024d; Gai et al., 2024). The reduced gel strength in A2/A2 milk is likely due to fewer protein-protein interactions and a more porous protein network, leading to cheese with distinct textural properties (Mendes, de Morais, & Rodrigues, 2019). Interestingly, at the end of the ripening period, conventional cheese is found to be softer and less cohesive, despite exhibiting the same level of proteolysis as A2/A2 cheese (Daniloski et al., 2024d). Although these results suggest that the structural differences in the cheese samples may be attributed to the single amino acid variation between β-casein A1 and A2, the differing levels of κ-casein and calcium in the milk types cannot be disregarded (Lucey, 2022). Conventional milk, with its higher κ-casein content, forms a greater number of bonds between casein micelles during acid gelation, resulting in firmer gels. Additionally, the higher calcium content, particularly ionic calcium, in conventional milk contributes to smaller casein micelles and enhanced rennet coagulation properties, further improving the texture and firmness of the final dairy products (Poulsen et al., 2013; Poulsen & Larsen, 2021).

5. Conclusions

The growing interest in A2/A2 dairy production, driven largely by consumer demand for perceived health benefits, presents both opportunities and challenges for dairy processors. While A2/A2 milk compared to conventional milk offers potential advantages, such as reduced digestive discomfort for some individuals, it also presents significant challenges in terms of processing. The structural differences between β-caseins A1 and A2 affect the stability of the casein micelle, leading to issues in acid gelation, rennet coagulation, and heat stability. For dairy processors, these challenges necessitate a deeper understanding of the behaviour of A2/A2 milk under different processing conditions. By tailoring processing techniques and potentially incorporating additional ingredients or steps, it is possible to produce high-quality dairy products from A2/A2 milk. However, these adjustments may require more precise control and could increase production costs. Ultimately, the ability to produce consistent, high-quality A2/A2 dairy products will depend on the successful integration of these insights into the dairy processing industry.

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