# A2 Milk

Subjects: Others

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Although milk consumption is increasing worldwide, in some geographical regions, its consumption has persistently declined. This fact, together with the increase in milk production prices, has caused both milk producers and the dairy industry to be immersed in a major crisis. Some possible solutions to this problem are to get people who do not currently consume milk to start drinking it again, or to market milk and dairy products with a higher added value. In this context, a type of milk called A2 has recently received attention from the industry. This type of milk, characterized by a difference in an amino acid at position 67 of the β-casein polypeptide chain, releases much smaller amounts of bioactive opioid peptide β-casomorphin 7 upon digestion, which has been linked to harmful effects on human health. Additionally, A2 milk has been attributed worse technological properties in the production of some dairy products. Thus, doubts exist about the convenience for the dairy industry to bet on this product.

Keywords: A2 milk ; β-casein ; β-casomorphin 7 ; lactose intolerance ; gastrointestinal discomfort

## 1. Introduction

Milk is regarded as one of the staples of Western diets because of its high nutritional value. It is the first food in the diet of mammals, providing all the energy and nutrients necessary for growth and development in their first periods of life [1]. Milk intake stops after weaning in all mammals except in humans, who continue their consumption in adulthood, not only as milk but also as dairy products. In recent years, the dairy sector has been immersed in a major crisis in Europe due to high price volatility, increments of production costs, and a recent deregulation process, due to the abolition of milk quotas [2][3]. Additionally, their consumption is being replaced in some consumer groups by plant-based milk substitutes, which are often presented as a healthier, more sustainable, and animal-friendly alternative to bovine milk [1].

In this context, the dairy sector needs to find ways to increase profitability [2]. One possible alternative is to incorporate milk into the diet of consumers who do not currently consume it. In this case, one of the major segments of the population that could increase the consumption of milk and dairy products are those who suffer adverse reactions after ingestion [4]. Digestive discomfort is mainly associated with lactose malabsorption, which affects approximately 65% of the adult population worldwide [5]. Lactose-intolerant individuals suffer diverse digestive symptoms after milk ingestion, such as abdominal pain, bloating sensation, stool frequency changes, and stool consistency changes [6]. Another option to increase dairy profitability is the search for differentiated products with a higher added value [7], such as milk or dairy products with associated benefits to human health. Food with health claims shows higher prices than those observed in high-volume food segments [8].

More than 95% of the proteins contained in cow's milk consist of caseins and whey proteins. Caseins represent approximately 80% of total milk protein, and whey proteins represent about 20%. Among bovine skim milk caseins, four different types have been described: α-casein S1 (ranging 12–15 g/L), β-casein (9–11 g/L), α-casein S2 (3–4 g/L), and κ-casein (2–3 g/L). Among other protein fractions in bovine skim milk, the most relevant content is for α-lactalbumin (0.6–1.7 g/L), serum albumin (0.4 m/L), immunoglobulins (0.5–0.8 g/L), lactoferrin (0.02–0.1 g/L), and the secretory component (0.02–0.1 g/L) [9].

These proteins have a major implication in milk production, the industry, and consumer health. β-casein occupies the second position in bovine milk caseins in terms of abundance, in addition to presenting many amino acids [10][11]. All types of caseins undergo modifications in their structures due to the substitution or exclusion of some amino acids of the peptide chain, thus generating genetic variants. When there is more than one structural variant encoded by a gene, it can be referred to as a polymorphism [12]. These genetic variants that affect protein structures cause changes in their characteristics, in addition to influencing milk production and technological applications for industrial use [13].

In recent years, a new type of cow's milk, named "A2 milk", has been introduced in the market. This type of milk was first commercialized in New Zealand and has since been gaining a presence in the markets of several countries [7]. A2 milk is characterized by being free of the A1 variant of β-casein: a protein that represents approximately 30% of the caseins in cow's milk [8]. By genotyping dairy cows and using semen from selected bulls, or genotyping and selecting only A2 calves for replacement, it is possible to obtain a dairy farm producing only A2-type milk [7]. The coding gene in β-casein synthesis is the CNS2 gene, for which 13 different allelic variants have been described (A1, A2, A3, B, C, D, E, F, G, H1, H2, and J) [14]. The A2 variant is present in the milk of many mammals, both in humans and in goats, sheep, and cows, while the A1 variant is only present in cattle [12].

The two most common variants of this protein are types A1 and A2, which differ by only one amino acid at position 67. In type A1 β-casein, there is an amino acid histidine at this position, whereas in type A2 protein, this histidine is replaced by a proline [15]. Thus, the original codon cytosine-cytosine-thymine (CCT), which forms the amino acid proline in the A2 variant, is modified to cytosine-adenine-thymine (CAT), which encodes the formation of histidine at position 67 of the β-casein polypeptide chain in A1 variants [16]. Dairy cow breeds show different β-casein patterns in their milk. Thus, the breed with the highest percentage of A2 alleles in Europe is Guernsey, with 92%, whereas A1 is the most common allele in other dairy breeds, such as Holstein (60%) or Ayrshire (60%) [17]. Currently, most milk marketed contains a mixture of A1 and A2 β-casein, which may be from A1/A2 heterozygous cows or from the milk mixture of A1/A1 and A2/A2 homozygous animals [18].

## 2. The A2 Milk Market

Although the worldwide consumption of milk and dairy products is constantly increasing and is expected to continue increasing over the next decade [19], milk consumption has decreased significantly since the 1970s in specific geographical areas, such the United States and the European Union [20][21]. Thus, the dairy industry has tried to be creative and develop new products to increase consumption. In 2003, the A2 Milk Company Limited emerged in New Zealand, commercializing both milk and dairy products (cheeses, yogurts, or creams) free of the A1 variant of β-casein. A2 milk strongly entered the market in this country and covered almost 10% of the milk market in Australia. In view of the possible benefits of A2 milk for human health, in addition to avoiding the negative effects of β-casein A1, many farmers around the world have switched to A2 milk production [22]. This successful market trend has spread to other geographic areas, such as North America, Europe, and China [20]. Consequently, other companies dedicated to the commercialization of semen for dairy farms have introduced the A2/A2 genotype in their sire directories as a characteristic of interest and added value for their animals [23].

The adoption of the A2 milk production system is complicated because not all farmers are willing to genotype their cattle to obtain 100% A2 milk. Generally, it is assumed that the Al allele brings better production performance characteristics in dairy cows than the A2 genotype [24], although some works did not find significant differences in milk production [25], and some studies even found that higher milk production rates are obtained with the A2 genotype [11][26]. Economically, genetic selection is not an excessive expense because genotyping tests are becoming cheaper [23]. However, the milk production process must be separated from that of milk coming from A1 cows. The cows must be milked separately, in separate tanks, with milk independently transported, brought to the factory separately, and manufactured without mixing or contaminating with A1 protein, as the risk of this happening is very high. Thus, it is necessary to have a large potential market and a milk price premium to make the whole process profitable. Many people could be interested in consuming only A2 milk, but only if its beneficial effects for human heath have been demonstrated by scientific evidence [7]. A recent work investigating consumers' awareness of A2 milk [21] revealed that the factors that condition consumers to buy these dairy products are price; origin; and quality certification, such as "organic" or "traditional". This same work revealed that the premium price that Italian consumers are willing to pay for A2 milk with respect to fresh lactose-free milk is approximately 20-euro cents/liter [21]. Other work [27] revealed that only 38% of a Brazilian group of consumers would pay an extra price for A2 milk with respect to conventional milk. The first dairy company commercializing A2 milk (The A2 milk company) claims that they pay a premium of around 5–7% to its farmer suppliers in New Zealand, Australia, and the United Kingdom [28].

A recent work [29] revealed that a high proportion of consumers did not know about A2 milk, and thus had never considered purchasing this type of milk. Moreover, a positive relationship was found between socioeconomic level and A2 milk awareness [30]. Therefore, it seems important to promote marketing strategies to improve awareness of A2 milk among less informed consumers and modify their buying behavior.

Additionally, in recent years, some legal problems have arisen from the adulteration of some milk marketed as A2 with other non-A2 milks. This addition of non-A2 milk to A2 milk represents fraud for consumers because they pay an extra price for this adulterated A2 milk, and they may be concerned about the consumption of mixed β-casein variant A1 [22]. This fact makes it necessary for the marketing of A2 milk at a premium price to be viable and for consumer authorities to implement good systems to control this possible fraud.

## 3. Physical and Technological Properties of A2 Milk

A recent work [31] investigating the sensory quality, color, and composition of A2 milk compared with A1 milk found that different genotypes did not affect the smell, taste, or general acceptance of the milk. However, some differences were found in the color. A2 milk showed color parameters closer to the gold standard for color, making it more appealing to consumers without artificial food coloring [31].

Milk protein composition is an important factor in the nutritional and technological characteristics of milk. The quantities and proportions of milk caseins and whey proteins have been identified as playing a major role in milk coagulation and curd firming processes [32]. Several studies have extensively assessed the effects of milk protein genetic variants on the composition and protein profile of milk [31][32][33] and its coagulation properties [31][32][33][34][35]. Milk caseins play an important role in the production of dairy products, such as in cheese manufacturing [36][37]. However, there are still few reports about the influence of A2 milk on dairy product manufacturing and consumer perceptions.

Because of the increasing trend in A2 milk consumption in some countries [38][39][40], some farmers and breeding associations began to increase the frequency of the β-casein A2 allele in dairy cattle populations without paying attention to the potential effect of this change on milk technological characteristics [29]. Nevertheless, it was revealed that due to their composition, A2 milk has a higher percentage of total proteins and fat than A1 milk [12]. A recent work found slight differences in the amino acid composition of A2 and A1 milk, showing that A2 milk had a higher amount of leucine that A1 milk but lower overall amino acid content [31]. A2 milk could also have worse technological characteristics to produce some dairy products, such as cheese or yogurt [29]. Therefore, producers should keep in mind that if its production is not for sale as drinking milk but as an ingredient for the manufacturing of dairy products, the better price they expect to obtain for A2 milk could remain without effect or even be considered a negative factor by the industry.

β-casein influences the gelation and coagulation of milk because it plays a central role in casein micelle formation. According to Nguyen et al. [39], A2 β-casein is less hydrophobic, more soluble, and has a higher chaperone activity than A1 β-casein. According to this, several authors [31][32][33][34][35][36][37][38][39][40][41][42][43] associated the A2 milk variant with poor rennet coagulation properties. A further study [29] also found that the A1 milk genotype was associated with the best cheese-making abilities with respect to A2 milk due to a slight worsening of the coagulation properties for A2 milk.

Other works [38] suggested that different genetic variants of β-casein could influence the distribution and balance of calcium in milk. Thus, such genetic variants could influence the assembly and structure of casein micelles [43], thereby affecting curd formation during cheese making (**Table 1**). Several authors [38][39][40][41][44] reported that the gelling process of A2 milk is slower during fresh cheese making. In addition, a more porous microstructure and finer protein strands were observed in the gel made from milk with β-casein A2, which cause a lower gel strength compared to the gel from β-casein A1 milk [38]. However, a group of consumers detected no differences at the organoleptic level when asked about the taste of two types of Brazilian cheeses made from A1 or A2 milk, and only in one case (Minas Frescal cheese) did consumers find the cheese made from A2 milk softer and creamier and less consistent, rubbery, and drier than A1 cheese [27]. From these results, it can be concluded that A2 milk is associated with higher digestibility and better gut physiology, and the dairy industry should favor this genotype in milk destined for fresh consumption. However, it is inadvisable to use milk containing only the A2 variant of β-casein for cheese production, as this is associated with a worsening of milk technological characteristics and, consequently, a less efficient cheese-making process [29].

Additionally, it was reported that A2 milk had lower fat globule diameters [45] and higher polyunsaturated fatty acids content [31] than A1 milk [45]. Milk fatty acids and fat globule size influence the physicochemical, nutritional, and sensorial properties of milk and milk products [45]. The morphometric characteristics and fatty acid composition of milk are strongly influenced by casein polymorphism, as shown by the significant differences found among casein haplotypes of milk. These results are of interest because the degree of differentiation in globule size influences renneting, cheese texture, color, flavor, and butter texture. With respect to fat % in A2 milk, some authors found higher rates of fat in A2 animals than in A1 genotype cows when produced by the same dairy cow breed and in the same ambient [11][24].

Other important processes for the dairy industry for which a different activity was reported from A1 and A2 milk are emulsion and foaming capacities, although the results reported are not entirely consistent. The A2 β-casein variant was associated with poorer foaming capacity compared to A1, which was due to a more extensive spread of β-casein A1 at the interface, facilitating the more rapid formation of a coherent interfacial layer than in A2 [46]. In contrast, it was also reported that milk with the β-casein A2 variant had better foaming properties than A1 milk [37]. The emulsion properties of milk containing different β-casein variants were compared by Darewicz and Dziuba [47]. They concluded that A2 milk was more efficient than A1 milk in the emulsion formation, but its emulsions were less stable than those formed with the A1 and B variants. In addition, the A1 and B variants have better ordered structures in the absorbed state than A2, which also contributes to differences in their emulsifying ability [47] (**Table 1**).

In addition to cow's milk, one work also investigated the allergenic and physicochemical properties of A2 goat's milk [48], finding that the physicochemical properties of the A2 β-casein fraction are similar to those of bovine whole casein.

**Table 1.** Differences in sensory characteristics and technological properties of A1 and A2 milk and dairy products.

| Parameters Investigated | Samples | Main Findings | Reference |
|---|---|---|---|
| **Chemical, protein profiles and rheological properties of milk and yogurt** | **Dried milk power obtained from raw A1A1 and A2 whole milk from Kiwi crossbred cows** | - A2 milk had higher free calcium concentration and better foaming formation than A1 milk.<br>- Yogurt prepared with A2 milk capacity had better porous microstructure and thinner protein strands than A1 milk-added yogurt. | [37] |
| **Triangular test, focus group test, temporal dominance of sensations, overall sensory acceptance, online questionnaire with 17-multiple test questions** | **Petit Suisse and Minas Frescal cheese made with both A1 and A2 bovine milks** | - Minas Frescal samples performed with A2 milk were softer and creamier than those made with A1 milk.<br>- No difference in overall acceptability was found for Petit Suisse cheese.<br>- Consumers reported that they did not read labels and the information about the type of milk frequently. | [27] |
| **Dinamyc rheological analysis, rennet coagulation time, maximum coagulum strength, and curd firming rate** | **Morning milk obtained from 892 individual Danish cows (456 Holstein-Friesians and 436 Jerseys)** | - A2 milk showed poorer coagulant properties than other tested milks. | [40] |
| **Rheological analysis** | **Morning milk collected from 1299 Danish Holstein, Danish Jersey, and Swedish Red cows** | - A2 milk showed poorer coagulant properties than other tested milks. | [42] |
| **Emulsion properties, surface excess, conformational analysis, and MALDI-TOF spectra** | **Whole casein from milk obtained from individual Jersey, Friesian-Holstein, and German Black and White cow breeds** | - β-casein B variant showed better emulsion-stabilizing properties than A2 and A1 variants. | [47] |
| **Interfacial and foaming properties** | **Purified milk protein preparations** | - A2 β-casein variant was associated with poorer foaming capacity compared to A1. | [46] |
| **Composition, allergenic properties, and physicochemical properties** | **Goat milk** | - The physicochemical properties of A2 β-casein fraction are similar to those of bovine whole casein. | [48] |

| Parameters Investigated | Samples | Main Findings | Reference |
|---|---|---|---|
| **Milk composition, cheese-making traits, and protein fraction identification** | **Individual milk samples collected from 1133 Holstein Friesian cows reared in 5 different herds** | - A2 milk showed higher β-lactoglobulin and α-lactalbumin, as well as a lower production of β-casein with respect to the A1 milk.<br>- Regarding milk cheese-making ability, the A2 genotype showed worst performance compared with the other genotypes, particularly with respect to the A1, with a higher rennet coagulation time. | [29] |
| **Milk composition, and capillary electrophoresis** | **Morning milk samples and tissue/blood samples were collected from 415 dairy cows (20 Danish Holstein, 22 Danish Jersey, and 392 Swedish Red)** | - Higher frequency of A1 milk, together with a decrease in A2 milk, could have positive effects on processing of cheese. | [32] |
| **Composition analysis** | **23,970 milk samples from 2859 Holstein cows** | - A2 milk showed worse milk coagulation time and curd firmness than B genotypes. | [35] |
| **Fat globule size, fatty acids profile** | **250 Holstein were defined for their genotypes** | - A2 milk showed most small fat globules and less big fat globules than other genotypes. | [45] |
| **Protein characterization, rheological analysis** | **Individual milk samples from 121 cows in mid lactation of the Swedish Red and Swedish Holstein** | - The β-casein A2 genotype was associated with inferior milk coagulation characteristics. | [44] |
| **Quantification of milk proteins, casein micelle size, milk fat globule size, milk coagulation properties, salts distribution** | **Individual morning milk samples from 99 Norwegian Red cattle cows** | - A1 milk showed better coagulation properties such as rennet coagulation properties and crud firmness than A2 milk. | [49] |
| **Milk protein characterization** | **Morning milk samples and blood samples of 1912 firt-lactation Holstein-Friesian cows** | - A2 genotype was associated with higher relative concentrations of β-casein, lower of αS2-casein, and with higher protein yield than A1 genotype. | [50] |
| **Milk protein and fat characterization** | **Milk and blood samples of 20,928 Ayrshire cows** | - Milk and protein production was highest for the β-casein A2 genotype, and fat percentage was highest for the A1 genotype. | [51] |
| **Milk composition, physicochemical analysis, gelation properties** | **Milk from genotyped 114 cows** | - A2 milk was associated with poor acid gelation properties. | [52] |

| Parameters Investigated | Samples | Main Findings | Reference |
| --- | --- | --- | --- |
| **Milk coagulation traits and protein composition** | **1042 multiparous Holstein cows** | - A2 milk was instead associated with poorly coagulating milk, higher protein content, and made milk less suitable for cheese making than other genotypes. | [53] |

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