Ann. Anim. Sci., Vol. 25, No. 1 (2025) 373–384 *DOI: 10.2478/aoas-2024-0092*

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COMPARISON OF THE QUALITY OF YOGHURT PRODUCED FROM MILK CONTAINING A1 AND A2 β-CASEIN

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Abstract

A2 milk, characterised by the presence of only the A2 β-casein genotype, has recently been of great interest due to its potential health benefits, which are the subject of intensive scientific research. From the dairy industry standpoint, it is crucial to ascertain its functional and technological attributes. The objective of this study was to compare two types of yoghurt (A1 and A2 yoghurts), produced respectively from cows' milk containing exclusively β-casein (β-CN) A1 (A1/A1) or A2 (A2/A2) genotypes, in terms of basic chemical composition, protein profile, amino acid composition, antioxidant capacity, sensory quality, acidity, instrumental texture and colour profiles, as well as rheological characteristics. The obtained results indicate that the β-CN polymorphism had no significant impact on the basic chemical composition, acidity, DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) or colour properties of the yoghurts. Nonetheless, the β-CN genotype did affect the textural, rheological, and sensory qualities of the acidic gels, with A2 yoghurt yielding gels exhibiting higher hardness, gumminess, improved consistency and viscosity, and overall sensory quality compared to the corresponding A1 treatment. These results indicate that A2 milk serves as a good raw material for fermented milk production, warranting further investigation.

Key words: A2 milk, β-casein genetic variant, yoghurt, texture, antioxidant capacity

Cow's milk typically contains 3.5% protein. Milk proteins are essential components of milk solids, contributing significantly to both nutritional value and technological aspects by forming the body and texture of various dairy products, particularly yoghurt and cheeses. However, milk proteins are not a uniform group but can generally be divided into three main classes: caseins (CNs), whey proteins (WPs) such as α-lactalbumins (α-LA), β-lactoglobulins (β-LG), bovine serum albumin (BSA), and immunoglobulins (IGs), as well as proteins associated with the fat globule membrane (MFGMPs) (Bär et al., 2019; Fox et al., 2015).

Caseins, the most abundant group, account for about 80% of the total protein concentration in cow's milk. They exist in four types (α_{s1}-CN, α_{s2}-CN, β-CN, and κ-CN) and various genetic variants. For instance, 13 genetic variants of β-casein (β-CN) have been identified so far in bovine milk, with A1 and A2 variants being the most frequent (Jeong et al., 2024; Radkowska, 2020). The difference between these two genetic variants is a single amino acid mutation within the peptide structure of β-CN. In the A2 genetic variant, position 67 in the peptide chain is occupied by proline (Pro), whereas in A1 milk β-CN, this position is occupied by histidine (His). Generally, the term A2 milk is restricted only to milk derived exclusively from homozygous A2A2 cows (milk containing exclusively A2 β-CN), whereas regular commercial cow's milk contains a combination of A1 and A2 variants of β-CN (milk produced by cows carrying A2A2, A1A1 or A1A2 β-CN genotypes) (Bodnár et al., 2018).

Research findings confirm that hydrolysis during human digestion of β-CN from A1 milk, unlike that from the A2 milk genetic variant, produces considerable amounts of β-casomorphin 7 (β-CM-7), a peptide that may negatively impact human health. It is hypothesized that this opioid-like peptide may be linked with an increased risk of ischemic heart disease, atherosclerosis, type-1 diabetes, sudden infant death syndrome, and neurological diseases such as autism and schizophrenia. Based on other research, consumption of A2 milk is suggested as a remedy for people suffering from cow's milk protein allergy and digestive disorders (Cieślińska et al., 2022; Park and Haenlein, 2021). However, there is no clear and definitive

*The work was financed from 501-100-511.

scientific evidence for a relationship between consumption of A1 milk or exposure to β-CM-7 and the aetiology of human non-communicable diseases, which suggests the need for further studies in this field (Cieślińska et al., 2022). The beneficial effect of consuming A2 milk has been demonstrated in clinical studies only in relation to improved digestive comfort and reducing symptoms of lactose malabsorption in individuals with diagnosed lactose intolerance (Milan et al., 2020).

Due to potential health benefits, A2 milk has been commercially produced and distributed in many countries, including the USA, Asia-Pacific countries, the Netherlands, Great Britain, Germany, France, Belgium and other countries (Park and Haenlein, 2021; Data Bridge Market Research, 2023). Despite the fact that health-related issues associated with the consumption of A2 milk are not entirely explained, scientific research has shown differences in structure and functionality between bovine A1 and A2 β-CN. Structural variability affects protein behaviour upon enzymatic treatment or acidic coagulation during both gastrointestinal digestion and technological processing of milk. These differences may be especially important in cheese and yoghurt production, potentially necessitating process adaptations based on the type of milk. According to some scientific reports, milk containing the A2/A2 β-CN genotype is less prone to rennet and acidic coagulation (e.g., needs more time to coagulate) and produces softer curd, which may be challenging for dairy producers (Nguyen et al., 2018 b; Bisutti et al., 2022). On the contrary, some reports show no significant differences between the β-CN A2/A2 milk and milk containing other combinations of β-CN variants regarding acid and rennet coagulation time (Hallén et al., 2009; Juan and Trujillo, 2022). However, it is worth emphasizing that discrepancies between results obtained by different authors may be due to variations in types and sources of bovine milk. For instance, milk of different cattle breeds may be analysed, comparison may involve the A2/A2 milk variant (A1-free milk) against bulk milk containing a combination of A1 and A2 β-CN genotypes, or A2/A2 milk may be compared with A1/A2 and/or A1/A1 milk. Additionally, research may employ fresh, UHT, or reconstituted milk or other processed milk.

Since the market for A2 milk in Europe is growing and is projected to rise at a CAGR of 17.2% by 2029 (Data Bridge Market Research, 2023) and the scientific information regarding its physicochemical and technological properties is still scarce, there is a need for further and comprehensive research and development in this field (Daniloski et al., 2022 b; Jeong, 2024). Furthermore, making yoghurt from A2 milk may be particularly beneficial, as yoghurt cultures, like A2 milk, also contribute to reducing symptoms of lactose maldigestion (Morelli, 2014). Additionally, studies conducted by Nguyen et al. (2018 a) suggest that starter bacteria during milk fermentation are capable of degrading β-CM-7 peptide. Therefore, the present study was established to compare selected physicochemical properties, including basic chemical composition, detailed protein profile, acidity, antioxidant capacity, instrumental texture and colour profiles, as well as rheological characteristics and sensory quality of yoghurts produced from milk containing the A2 β-CN variant (hereinafter referred to as A2 yoghurt) or the A1 β-CN genotype (A1 yoghurt) derived from Simmental cows during two periods (in June and September).

Material and methods

Material

The fresh raw milk intended for yoghurt production came from the Experimental Station of the National Research Institute of Animal Production in Odrzechowa, which is the largest facility for breeding Simmental cows in Poland. In the first stage of the research, identification of dairy cows was carried out based on the β-casein variant. The identification was performed on 560 cows. Genetic identification was done using the Allelic Discrimination Assay (ADA) method with fluorescently labelled TaqMan MGB probes (Applied Biosystems), a real-time polymerase chain reaction (Real Time PCR) method, at the Molecular Genetics Laboratory of the National Research Institute of Animal Production in Balice.

In the next stage of the research, cows identified as carrying A1A1 and A2A2 β-CN genotypes in their first and second lactation and at a similar stage of lactation (50–150 days of lactation), were selected. The cows were kept in a free-stall barn and fed the same way using the TMR feeding system according to nutritional standards. They were provided with identical care, maintenance, and proper welfare conditions.

Milk samples from Simmental cows carrying A1A1 (further referred to as A1 milk) and A2A2 (further referred to as A2 milk) β-CN genotypes were collected twice a year during milkings in June and September. The milk was cooled to a temperature of 4°C and delivered to the laboratory within approximately 3 hours. The milk, placed in special self-sealing vent bags, was transported in a compressor refrigerator, which allowed for maintaining and controlling the temperature. The collected milk samples were used to produce yoghurts in laboratory conditions at the Department of Animal Product Technology of the University of Agriculture in Krakow (Kraków, Poland), which were then subjected to analyses.

Direct-Vat-Set (DVS) type lyophilized yoghurt culture (YC-X11; composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) from Chr. Hansen (Hoersholm, Denmark) was used for milk inoculation. Before use, a tenfold amount of the required starter culture was weighed, 10 mL of sterile peptone water was added, mixed, and left for 15 min to hydrate. The milk was inoculated with 1 mL of the prepared starter culture solution. The final concentration of DVS yoghurt culture was 0.03 g in 1 L of milk.

Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was purchased from Fluka (Buchs, Switzerland, Copenhagen, Denmark and Madrid, Spain), whereas 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were purchased from Sigma-Aldrich (Steinheim, Germany and Buchs, Switzerland). Protein standards for SDS-PAGE, i.e. lactoferrin (LF), bovine serum albumin (BSA), immunoglobulin G (IgG), α-casein (α-CN), β-casein (β-CN), κ-casein (κ-CN), β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

All other chemicals used were of analytical reagent grade.

Production of yoghurts

Fresh, raw milk (A1 and A2 separately) intended for yoghurt production (approximately 2 L) was heated to 40–45 °C and centrifuged using a milk separator (LW-G24E milk separator – Spomasz, Gniezno, Poland) to achieve 2% fat content (total solids were not standardized). It was then homogenized twice using an Armfield FT9 milk homogenizer (Ringwood, England) at 65°C and 6 MPa, followed by pasteurization at 85°C for 15 min in kettles. Subsequently, the milk was cooled down to 43°C, inoculated with yoghurt culture (0.03 g/L milk) and poured into 200 mL sterile glass jars. The incubation was carried out at 43°C until the final pH reached 4.6–4.8 (approximately 4 h). The yoghurts were then cooled to 20°C in an ice water bath and stored in the refrigerator at 4°C prior to analyses.

Analytical methods

Chemical composition

Analyses of the basic chemical components of the yoghurts were performed only on fresh yoghurt samples and comprised determination of total solids (TS), fat (F), protein (P), and ash (A) concentrations (PN-75/A 86130). The content of carbohydrates (lactose) was calculated from the equation:

$$C\ (\%) = TS\ (\%) - F - P - A \qquad \text{(Eq. 1)}$$

Determination of protein fractions by SDS-PAGE

The separation of protein fractions was carried out using the SDS-PAGE gel electrophoretic method. For this purpose, 0.3 mL of yoghurt was dissolved in 1 ml of water, and then 40 µL of reducing buffer (0.5 M Tris-HCl at pH 6.8, glycerol, 10% SDS, 0.5% bromophenol blue, β-mercaptoethanol, deionized water) was added to 20 µL of the diluted sample, and heated at 95°C for 4 minutes. The prepared sample was applied in 5 µL aliquots to a polyacrylamide gel. Milk protein standards, i.e. lactoferrin (LF), bovine serum albumin (BSA), immunoglobulins (Igs), α-casein (α-CN), β-casein (β-CN), κ-casein (κ-CN), β-lactoglobulin (β-LG) and α-lactalbumin (α-LA), were placed in the end wells of the gel in the same volumes. The separation was conducted at a current voltage of 100 V on a 12% SDS-PAGE polyacrylamide gel. The separation was carried out using the BIORAD Mini Protein 3 Cell device (BioRad Laboratories Inc., Hercules, CA, USA) according to the methodology described by Laemmli (1970). The obtained gels were stained in a Coomassie R 250 solution (0.25 g L^{-1} Coomassie Blue R-250 (BioRad Laboratories Inc., Hercules. CA, USA), 40% (v/v) methanol, 7% (v/v) acetic acid and deionised water), and after de-staining in a buffer solution prepared by mixing 40% (v/v) methanol, 7% (v/v) acetic acid and deionised water, they were scanned and subjected to quantitative analysis using the Gelscan 2.0 electrophoretic gel analysis program by Kucharczyk (Warsaw, Poland).

Amino acids (AA) analysis by HPLC method

The amino acid (AA) profile determination was conducted using the reversed-phase liquid chromatography method employing the AccQ-Tag analytical kit from Waters (Milford, MA, USA). Approximately 30 mg of the sample was hydrolysed with 4 mL of 6 M HCl (POCH, Poland) with the addition of 15 µL of phenol (Sigma Aldrich, USA) at the temperature of 110°C for 24 h. The sample was sealed in a nitrogen atmosphere. The resulting hydrolysate was filtered through syringe filters (0.45 µm) and then dried using nitrogen. The prepared sample, after appropriate dilution, was subjected to the derivatization procedure according to Waters' recommendations. For this purpose, 10 µL of the sample was mixed with 70 µL of borate buffer (pH 8.2–9.0), and then 20 µL of 6-aminoquinolyl-*N*-hydroxysuccinimidylcarbamate (AQC) acetonitrile solution (3 mg/mL) was added to the mixture. The standards (Waters, USA) were treated using the same procedure.

Chromatographic separation was performed using a Dionex Ultimate 3000 HPLC chromatograph (Thermo Scientific, Germering, Germany) equipped with a gradient four-channel LPG-3400 SD pump, WPS 3000 TSL autosampler, and FLD-3400RS four-channel fluorescence detector. A Nova-Pak C18, 4 µm column (150×3.9 mm) from Waters was used for analysis. The separation temperature was 37°C. Elution was carried out in a two-component gradient at a flow rate of 1 mL/min; eluent A: acetic-phosphate buffer (pH 5.2), eluent B: acetonitrile/water (60:40). The gradient was programmed as follows: 0 min – 100% A, 0.5 min – 98% A, 15 min – 93% A, 19 min – 90% A, 32 min – 67% A, 33 min – 67% A, 34 min – 0% A, 37 min – 0% A, 38 min – 100% A, 64 min – 100% A, 65 min – 0% A. The following detection parameters were applied: excitation wavelength 250 nm, emission wavelength 395 nm. Quantitative analysis was performed using a single-point calibration (utilizing an analytical standard with a concentration of 100 pmol each). Data processing was done using Chromeleon 7.0 software.

Titratable acidity and pH

Active acidity (pH) in yoghurt samples was measured using an Elmetron (Zabrze, Poland) CP-411 pH-meter.

Titratable acidity was evaluated according to the Polish Standard (PN-A-86061:2002) and expressed as % lactic acid.

Antioxidant capacity – FRAP and ARP

The antioxidant capacity of the obtained yoghurts was evaluated using two methods: ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity (expressed as ARP – anti-radical power), following the procedure described in detail by Najgebauer-Lejko et al. (2011). The results of the FRAP analysis were reported as mM Fe^{2+}/kg, while ARP was expressed as mM TE (Trolox equivalent)/kg of the sample.

Texture – double compression test

The textural properties of the obtained yoghurts were measured using a TA-XT Plus texture analyser (Stable Micro Systems, Haslemere, Surrey, UK). Directly before analysis, the undisturbed yoghurt samples in the original glass jars (inner diameter of 45–55 mm, sample height of 50 mm) were removed from the refrigerator (4 °C). The test was performed using a plastic cylindrical probe (diameter: 50 mm, height: 5 mm), which penetrated the sample to a set depth of 15 mm at a speed of 1 mm·s^{-1}. Penetration was repeated twice. Based on the force vs. time function, the following parameters were calculated using the appropriate software: hardness (maximum force of the first compression cycle; N), adhesiveness (work measured during the withdrawal of the probe from the sample in the first compression cycle; |N·s|), cohesiveness (the ratio of the second compression to the first compression positive force area; dimensionless value), and gumminess (multiplication of hardness and cohesiveness, N·s).

Rheological studies

The rheological properties of the yoghurts were measured using a HAAKE iQ rotational rheometer (Thermo Scientific, Karlsruhe, Germany). A concentric cylinder measuring system CC 25DIN was applied. The distance between the inner and outer cylinder (ring gap) was set as 1.06 mm. Measurements were carried out using the CR (control rate) method in the range of 10–300 s^{-1}. The pattern of applying the sample was as follows: mixing the sample with a spoon by making 10 turns to the right and 10 turns to the left. The volume of the analysed sample was 16 mL. The sample was relaxed in the measuring geometry for 600 s at 10°C. Subsequently, the samples were subjected to a variable shear rate treatments in the following order: 1. increasing shear rate in the range of 10–300 s^{-1} in 90 s (momentum curve); 2. maintaining constant shear rate 300 s^{-1} for 10 s (structure destruction) and 3. decreasing shear rate from 300 to 10 s^{-1} shear rate in 90 s (return curve).

Based on the measured values of shear stress τ [Pa] and the set values of shear rate γ [s^{-1}] flow curves were plotted, and the rheological parameters of the analysed yoghurt samples were computed using the rheological model of Herschel-Bulkley, described by the following equation:

$$\tau = \tau_0 + k \cdot \gamma^n \quad \text{(Eq. 2)}$$

where: τ is the shear stress (Pa), τ_0 is the yield stress (Pa), k is the consistency coefficient (Pa·s^n), γ is the shear rate (s^{-1}), and n is the flow behaviour index (–).

The area of the hysteresis loop and apparent viscosity (η) for each sample were also calculated at the different shear rates. Viscosity curves were plotted as a function of the calculated apparent viscosity (η) values and the set values of shear rate γ [s^{-1}]. Initial apparent viscosity was determined for the shear rate of 10 s^{-1}, and final viscosity for 300 s^{-1}. The yield point was determined empirically as the intersection point of two tangents drawn to the curve in a doubly logarithmic coordinate system of strain-stress dependence γ(τ).

Instrumental colour measurement

Colour reflectance measurement was conducted employing a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan), using standard illuminant and D65/10°C. The following CIE*Lab** parameters were assessed: *L** for lightness on a scale from 0 (black) to 100 (white), *a** representing negative and positive values indicating green and red colour coordinates respectively, and *b** representing negative and positive values indicating blue and yellow colour coordinates, respectively. Additionally, hue angle (h) and chroma (C) indicating colour saturation, were also calculated. Each sample was warmed to 20°C and thoroughly mixed with a spatula before measurement.

Sensory quality

The sensory quality assessment was conducted in accordance with PN-ISO 6658 (1998) using a 5-point scale (from 1 – very bad to 5 – excellent) by a trained panel of 15 judges recruited from the academic staff and students. The sensory attributes evaluated for the yoghurts included colour, appearance, consistency, odour, and taste. The overall preference, referred to as a total score, was calculated taking into consideration the appropriate indexes of importance for each quality attribute (0.10; 0.15; 0.25; 0.15; and 0.35, respectively).

Experimental design and statistical analysis

The analyses were performed in four replicates (2 batches × 2 repetitions). The results were expressed as means ± SD. Evaluation of the basic chemical composition, gel electrophoresis, amino acid profile and sensory quality were performed on fresh yoghurt samples and subjected to t-test. Storage tests, including determination of acidity, antioxidant properties, textural studies and colour analysis, were carried out on the fresh yoghurt samples and after 3 weeks of storage at 4°C, and statistically analysed using two-way ANOVA (yoghurt type: A1 or A2, and storage time were the variability factors). Where

applicable, the significance of differences between mean values was estimated on the basis of Duncan's post-hoc test. Significant differences between mean values were established at $P \leq 0.05$. Statistica 13.3 software was used for statistical analyses (TIBCO Software Inc., Palo Alto, CA, USA).

Results

Chemical composition and protein characterisation

The basic chemical composition of A2 and A1 raw milk samples, as well as yoghurts made from them, is presented in Table 1. Generally, there were no statistically significant differences observed in total solids (TS), non-fat milk solids (NFMS), or the concentration of each analysed chemical component, either in milk samples or derived yoghurts, concerning the milk β-CN genetic variant.

The main protein compounds in both yoghurt types were caseins (CNs), which accounted for about 70–75% of the total protein, with the most abundant being α-CN (Table 2, Figure 1). In addition to CNs and serum proteins, a lower-molecular-weight fraction of peptides (approximately 6.5 kDa) and three fractions of unknown compounds (P1, P2, P3) not found in the milk samples, with molecular masses greater than β-LG but smaller than κ-CN, were detected. Immunoglobulins, which were present in the milk samples, were not identified in the yoghurts. Based on the results of electrophoresis, a decrease in the level of κ-casein and whey proteins, such as β-LG and α-LA, was observed in the yoghurts compared to the milk. This was accompanied by an increase in the quantity of the low molecular weight fraction of peptides. Furthermore, results indicate that A2 yoghurts contained a higher share of these peptides and a lower share of β-CN than A1 yoghurts.

Among all amino acids, glutamic acid (Glu) was dominant and accounted for 19.9–22.8% of total protein, although A2 yoghurt contained a significantly higher share of this compound than A1 yoghurt (Table 3). Both yoghurt treatments also differed in terms of the share of isoleucine (Ile), leucine (Leu) and phenylalanine (Phe) in their amino acid profile, with A1 yoghurt being more abundant in these amino acids.

Acidity and antioxidant capacity

No differences in titratable acidity or pH were found between yoghurts produced from both genetic variants of milk β-CN (Table 4). In each group, a significant decrease in pH and an increase in titratable acidity were noticed for yoghurts during storage due to the post-acidification effect.

Moreover, the genetic variant of β-CN in milk also had no influence on the antioxidant capacity of yoghurts measured as anti-radical power (ARP) against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical or ferric reducing antioxidant power (FRAP). Additionally, neither analysed antioxidant characteristic exhibited any significant variations during the three-week refrigerated storage of yoghurts (Table 4).

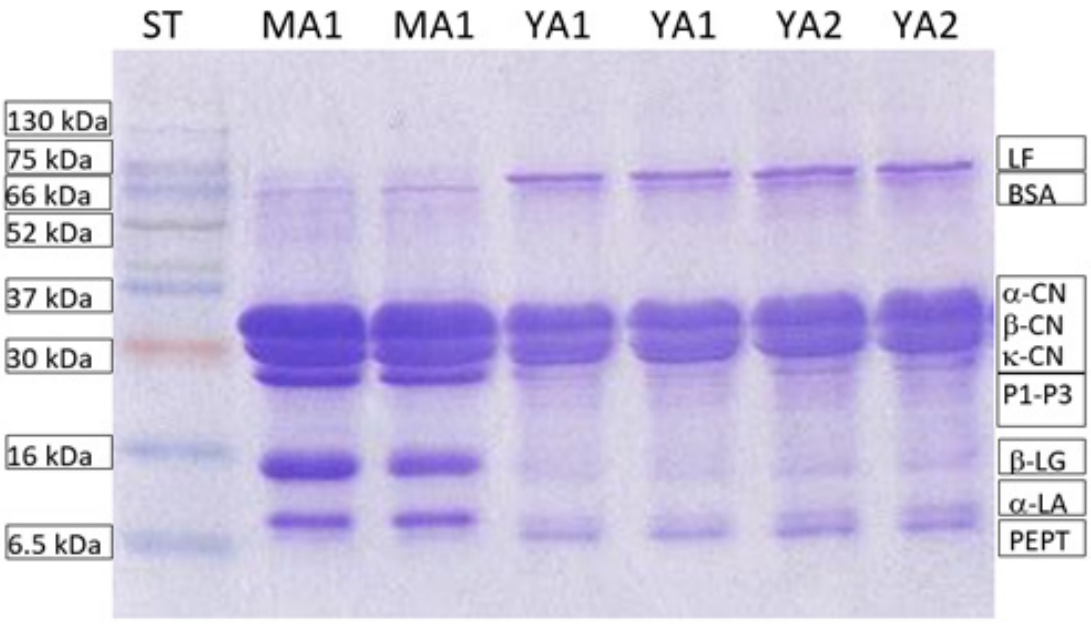

Figure 1. Electrophoretic patterns (SDS-PAGE) of milk and yoghurts prepared from milk of different β-CN genetic variants (ST – standards; MA1 – milk of A1/A1 β-CN genetic variant; YA1 – yoghurt from A1/A1 milk; YA2 – yoghurt from A2/A2 milk)

Table 1. Basic chemical composition of A1 and A2 milk and yoghurts (g/100 g; x ±SD, n=4)

Component	Milk		Yoghurt	
	A1	A2	A1	A2
TS	13.98 a±0.39	14.00 a±0.28	11.00 a±0.28	11.12 a±0.26
NFMS	8.75 a±0.24	8.73 a±0.31	9.13 a±0.12	9.25 a±0.01
Protein	3.43 a±0.11	3.59 a±0.07	3.63 a±0.28	3.66 a±0.20
Fat	5.24 a±0.20	5.28 a±0.10	1.87 a±0.20	1.89 a±0.16
Ash	0.7352 a±0.0110	0.7325 a±0.0096	0.8002 a±0.0081	0.7910 a±0.0233
Lactose	4.58 a±0.14*	4.41 a±0.23*	4.70 a±0.23*	4.79 a±0.25*

TS – total solids; NFMS – milk solids non-fat; *Lactose = NFMS – protein – ash.

a – the same letter denotes statistically insignificant differences between mean values within the respective characteristic and type of product (milk or yoghurt) at P≤0.05.

Table 2. Protein profile of A1 and A2 yoghurts (x ±SD, n=4)

Protein fraction	Yoghurt type	
	A1	A2
Lactoferrin	9.10 a±0.19 (+7.65)	9.37 a±0.36 (+8.20)
Bovine serum albumin	2.65 a±0.16 (+0.72)	3.50 a±0.54 (+1.96)
Immunoglobulins	ND (–1.15)	ND (–1.26)
α-casein	41.00 a±0.89 (+3.42)	40.06 a±0.10 (–0.24)
β-casein	29.06 b±0.93 (+6.09)	23.12 a±1.51 (+2.08)
κ-casein	4.88 a±1.19 (-6.25)	6.70 a±0.19 (-6.25)
P1	2.01 a±0.52	2.76 a±0.80
P2	1.24 a±0.01	1.18 a±0.02
P3	1.37 a±0.32	0.91 a±0.02
β-lactoglobulin	2.19 a±0.13 (–12.13)	2.70 a±0.23 (–11.43)
α-lactalbumin	2.43 a±0.47 (–4.84)	2.71 a±0.20 (–3.49)
Peptides	3.83 a±0.57 (+1.63)	6.84 b±0.06 (+5.42)

a, b – values in rows with different letters differ significantly (P≤0.05); P1, P2, P3 – unidentified protein fractions; the changes relative to raw milk samples (A1 and A2, respectively) are given in parentheses.

Table 3. Amino acid profile of A1 and A2 yoghurts (x ±SD, n=4)

Amino acid (% protein)	Yoghurt type	
	A1	A2
Asp	7.44 a±0.07	7.60 a±0.08
Ser	4.99 a±0.19	4.96 a±0.27
Glu	19.90 a±0.68	22.84 b±0.14
Gly	1.91 a±0.06	1.85 a±0.06
His	2.68 a±0.08	2.51 a±0.19
Arg	3.51 a±0.08	3.52 a±0.18
Thr	4.48 a±0.50	4.59 a±0.04
Ala	4.42 a±0.41	4.19 a±0.73
Prol	8.84 a±0.03	8.80 a±0.18
Cys	0.34 a±0.00	0.45 a±0.06
Tyr	4.76 a±0.08	4.78 a±0.03
Val	6.19 a±0.10	5.95 a±0.03
Met	2.89 a±0.04	2.74 a±0.04
Lys	8.07 a±0.20	7.73 a±0.14
Ile	5.23 b±0.09	4.88 a±0.04
Leu	9.46 b±0.05	9.00 a±0.06
Phe	4.80 b±0.05	3.60 a±0.09

a, b – values in rows with different letters differ significantly (P≤0.05).

Table 4. Acidity and antioxidant capacity of fresh (0w) and stored (3w) yoghurts produced from A1 and A2 milk types (x ±SD, n=4)

Characteristic	A1 yoghurts		A2 yoghurts	
	Fresh (0w)	Stored (3w)	Fresh (0w)	Stored (3w)
pH	4.45 b±0.06	4.21 a±0.02	4.46 b±0.04	4.21 a±0.03
Titratable acidity (% lactic acid)	0.84 a±0.03	0.96 b±0.03	0.82 a±0.04	0.92 b±0.04
FRAP ($mMFe^{2+}$/kg)	0.84 a±0.14	0.83 a±0.05	0.78 a±0.08	0.73 a±0.17
ARP (mMTE/kg)	0.19 a±0.09	0.17 a±0.04	0.17 a±0.08	0.20 a±0.06

TE – trolox equivalents; ARP – anti-radical power, FRAP – ferric reducing antioxidant power.
a, b – values in rows with different letters differ significantly (P≤0.05).

Table 5. Textural and rheological characteristics of A1 and A2 yoghurts (x ±SD, n=4)

Characteristic	Yoghurt type	Storage time (week)	
		0w (fresh)	3w (stored)
Hardness (N)	A1	0.806 a±0.089	0.913 a±0.062
	A2	0.909 a±0.084	1.059 b±0.100
Adhesiveness (|N×s|)	A1	2.222 a±0.677	2.791 a±0.448
	A2	2.629 a±0.390	2.861 a±0.979
Cohesiveness (–)	A1	0.394 a±0.012	0.407 a±0.027
	A2	0.379 a±0.011	0.402 a±0.016
Gumminess (N)	A1	0.317 a±0.034	0.371 b±0.016
	A2	0.344 ab±0.030	0.424 c±0.023
Hysteresis loop area ($Pa \cdot s^{-1}$)	A1	6.800 a±0.136	6.747 a±0.069
	A2	8.799 c±0.159	8.488 b±0.082
k ($Pa\ s^n$)	A1	4.199 a±0.676	8.642 b±0.780
	A2	8.851 b±0.377	19.560 c±0.629
n	A1	0.390 c±0.029	0.258 b±0.015
	A2	0.201 b±0.088	0.084 a±0.036
R^2	A1	0.973 b±0.011	0.994 c±0.002
	A2	0.975 b±0.013	0.941 a±0.005
Initial η (Pas)	A1	5.273 a±0.197	5.497 a±0.161
	A2	6.235 b±0.180	6.342 b±0.062
Final η (Pas)	A1	0.207 a±0.005	0.218 b±0.005
	A2	0.239 d±0.005	0.228 c±0.003
τ_0 (Pa)	A1	22.563 a±0.729	31.753 c±0.615
	A2	28.127 b±0.847	37.960 d±0.893

a, b, c – different letters denote statistically significant differences between mean values within the selective characteristic (P≤0.05).

Table 6. Sensory properties of A1 and A2 yoghurts (x ±SD, n=4)

Sensory characteristic	Yoghurt type	
	A1	A2
Colour	5.00 a±0.00	5.00 a±0.00
Appearance	3.68 a±0.70	3.96 a±0.54
Consistency (body and texture)	4.46 a±0.46	4.82 b±0.37
Odour	4.68 a±0.42	4.89 a±0.29
Taste	4.50 a±0.59	4.68 a±0.42
Total score	4.44 a±0.26	4.67 b±0.24

a, b – values in rows with different letters differ significantly (P≤0.05).

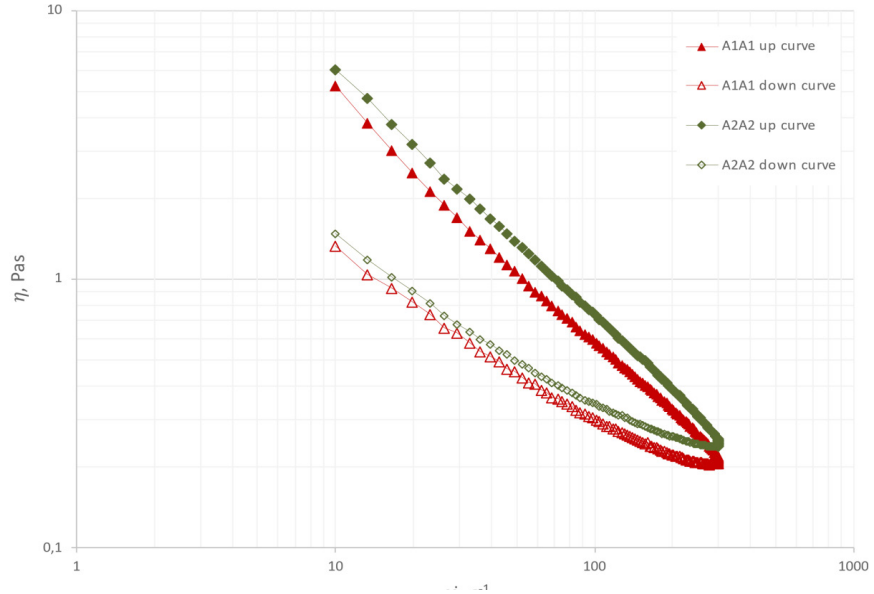

Figure 2. Viscosity curves of fresh A1 (red curve) and A2 yoghurts (green curve)

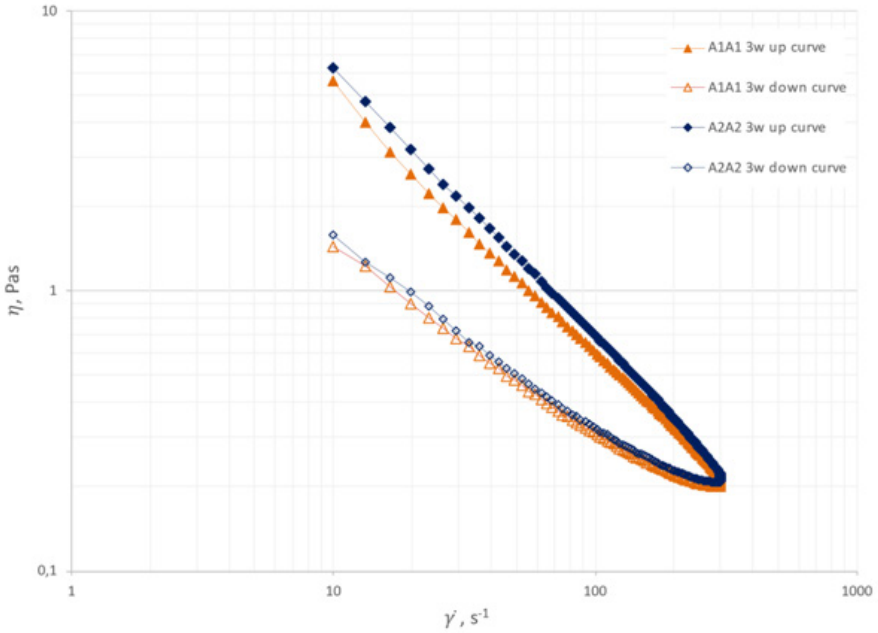

Figure 3. Viscosity curves of stored A1 (orange curve) and A2 (blue curve) yoghurts

On the other hand, data received from the textural and rheological studies indicate significant differences in yoghurt gel physical properties between both yoghurt treatments (Table 5, Figures 2 and 3). Fermented milks produced from A2 milk were characterised by higher hardness and gumminess; however, significant differences were noticed only after storage. An increment of the values of the aforementioned textural characteristics was also observed after three weeks of cold storage. A2 yoghurts were characterised by a firmer and more viscous structure when compared to the A1 variant, as indicated by higher values of the consistency coefficient (*k*), initial and final apparent viscosity, and yield stress (τ_0). The flow behaviour index (*n*) is a measure of the deviation of the rheological characteristics of the tested samples from the Newtonian fluid. Small values of the coefficient *n* indicate that the rheological properties of the tested yoghurts were significantly different from those of Newtonian fluids. Out of the two tested types of yoghurts, the A2 treatment exhibited a greater deviation from the properties of a Newtonian fluid as indicated by the lower values of the *n*-index.

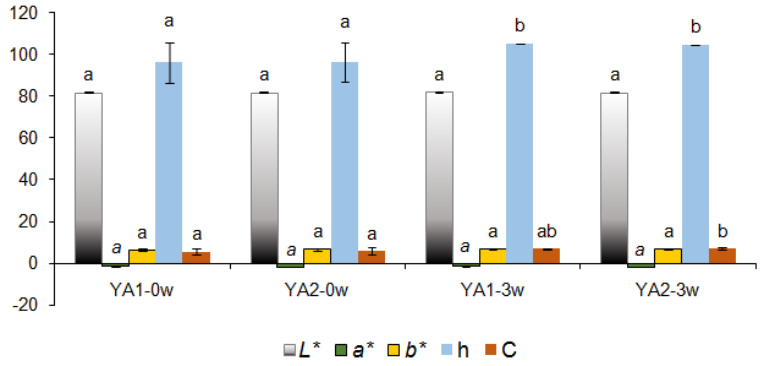

Figure 4. Colour characteristics of A1 and A2 yoghurts (YA1-0w – fresh A1 yoghurt; YA2-0w – fresh A2 yoghurt; YA1-3w – stored A1 yoghurt; YA2-3w – stored A2 yoghurt; a, b – different letters denote significant differences between results for a given parameter at P≤0.05)

The direction of changes of the final viscosity of fresh and stored yoghurt samples depended on the milk β-CN genetic variant used for production, as viscosity of the A1 yoghurts increased after storage, whereas in the case of A2 milk, lower values were measured after cold storage. Significantly higher values obtained for hysteresis loop area suggest that the structure of A2 yoghurts was also more prone to shear thinning.

The course of the viscosity curves presenting changes in apparent viscosity during increased shear rates and subsequent decreased shear rates (Figure 1) is typical for shear-thinning, pseudoplastic materials, and all analysed treatments fitted well to the Herschel-Bulkley model as indicated by the R^2 values.

On the other hand, A2 and A1 yoghurts did not differ significantly in terms of instrumentally measured colour attributes (Figure 4). The values of *L**, *a** and *b** coordinates (negative and positive, respectively) suggest that samples were light, slightly greenish, and yellowish. Hue angle and saturation of colour tended to increase during storage, whereas other colour characteristics did not change significantly.

The results of sensory analysis (Table 6) confirm the observations made during instrumental testing of texture and colour. Panellists gave higher ratings for consistency to A2 yoghurts. As the proper body and texture of the yoghurt gel is a very important feature for the total sensory quality of yoghurt, it also affected the higher total score of A2 yoghurt. On the other hand, there was no difference in colour between both yoghurt types which was also demonstrated in the instrumental colour analysis.

Discussion

Our study showed that neither yoghurt type, produced from milk containing different genetic variants of β-casein (β-CN), varied as regards basic chemical composition, including protein content, but differed in the more detailed composition of the protein fraction. Similarly to our results, de Vitte et al. (2022) and Nguyen et al. (2018 b) also reported that there are no significant differences in the basic chemical composition between A1/A1 and A2/A2 milk variants.

Bisutti et al. (2022), while studying the effect of β-CN polymorphism in milk of Holstein-Friesian cows on protein profile and suitability for cheese production, observed that the β-CN A1/A1 variant was associated with a lower concentration of α_{s2}-CN as well as certain whey proteins, i.e. β-lactoglobulin (β-LG) and α-lactalbumin (α-LA), and a higher production of β-CN when compared to the A2/A2 genotype. In our study, we also found a higher share of β-CN in the protein profile of yoghurts produced from A1/A1 milk. Additionally, A2 yoghurts in the current research were also characterised by a relatively higher share of whey proteins; however, the differences were not significant. It is also worth highlighting that the protein profile of milk is believed to undergo some changes under the action of lactic acid bacteria during yoghurt production. Generally, both yoghurt bacteria produce enzymes that are capable of hydrolysing α-, β- and κ-CNs in a different sequence of hydrolysis, but caseinolytic activity is mainly attributed to lactobacilli (Tamime and Robinson, 1999). In the current study, proteolytic activity of yoghurt bacteria in milk during incubation and subsequent storage resulted in the degradation of κ-CN and the formation of peptides with different masses (P1–P3 fractions ~16-30 kDa and peptide fraction ~6.5 kDa). The proteolytic changes seem to be more advanced in A2 yoghurt, as it contained a higher share of small molecular peptides. However, degradation of κ-CN in A2 yoghurt was not greater with respect to the A1 treatment. This observation is in accordance with the results presented by Wang et al. (2022), who determined a higher level of proteolysis in yoghurt produced from A2 milk than in yoghurt from normal milk. However, it should be mentioned that the observations made based on the SDS-PAGE results should be considered preliminary and need to be confirmed by more exact quantitative methods.

In the study of de Vitte et al. (2022), milk of various β-CN genotypes did not differ in the general chemi-

cal composition, but their amino acid composition was influenced by the genetic variant. However, in contrast to the results of our study, the authors observed significantly higher Leu content and simultaneously lower concentrations of Asp, His, Lys, Met, and Val in A2/A2 milk compared to that of the A1/A1 β-CN milk variant. This may result from the fact that other factors may contribute to amino acid composition, such as different breeds of cows, housing conditions, and nutrition patterns. Furthermore, lactic acid bacteria (LAB) during yoghurt fermentation may also utilise some nitrogen compounds to produce growth factors. LAB are known to possess a system of both proteinases and peptidases, which enable them to hydrolyse caseins to oligopeptides, then to smaller peptides and amino acids which are subsequently subjected to further degradation (Shihata and Shah, 2000). Among yoghurt starter cultures, lactobacilli exhibit higher proteolytic activity and are able to produce peptides and free amino acids, which are in turn utilised by *S. thermophilus,* which requires glutamic acid, histidine, methionine, cystine, valine, leucine, and tyrosine to grow (Beshkova, et al., 1998). It is postulated that differences in amino acid composition of milk proteins between A2 and A1 yoghurts not only result from the differences occurring in both milk types, as suggested by other authors, but also from different proteolytic patterns under the action of lactic acid bacteria in both treatments. Further studies are needed to confirm this thesis in fermented milks using different starter cultures, as most of the published reports deal with the aspect of enzymatic digestion *in vivo* or during cheese production.

Differences in the protein fractions between A2 and A1 genetic variants did not affect the lactic acid fermentation, as similar values of active and potential acidity were achieved simultaneously for both yoghurt types. However, they significantly influenced the structure and consistency of the obtained yoghurt gels, as reflected in higher values of hardness, gumminess, and viscosity in instrumental testing, and higher scores for consistency in sensory assessment for A2 yoghurt when compared to the A1 treatment. These results are surprising because they do not align with most literature data. For example, according to Daniloski et al. (2022 a), who examined the gelation behaviour of milk, A2/A2 skim milk was associated with worse acid gelation properties than corresponding A1/A1 and A1/A2 milk genotypes. Acid gels obtained from A2/A2 milk were characterised by a less dense microstructure, larger pore size, lower elastic modulus, water holding capacity, and gel permeability than other analysed milk genetic variants. The authors explain this with the less ordered secondary structure of β-casein and/or lower calcium and κ-casein contents. However, in the cited research, milk was not acidified using starter cultures of bacteria, but gelation was induced using glucono-δ-lactone. Another reason for the discrepancy between results may be due to additional factors, such as extrinsic, farm factors or those connected with casein haplotype percentages of a herd. For example, Dantas et al. (2023) mentioned that there are also different genotypes of other casein forms in milk, such as κ-CN and αs1-CN, that may influence milk technological properties.

Nguyen et al. (2018 b) reported that the use of reconstituted milk containing β-CN phenotype A2/A2 for yoghurt production delayed the acid gelation of milk and resulted in a more porous result, composed of thinner protein filaments and a weaker structure compared to β-CN A1/A1 milk. Additionally, Giribaldi et al. (2022), in their review concerning A2 milk quality, suggested that A2/A2 β-CN milk may produce softer, less dense, and more prone to deformation yoghurt gels. However, both reports are contradictory to our observations. Nevertheless, recent research of Wang et al. (2022) confirmed our results indicating that A2 milk produces firmer and more viscous gels upon lactic fermentation than normal milk, additionally the authors highlighted the lower susceptibility to spontaneous syneresis and smoother microstructure of the A2 yoghurt observed under an inverted fluorescence microscope. However, the authors used UHT bovine milk purchased in a local store for yoghurt production. The study of Ketto et al. (2017) on the influence of protein polymorphism in milk from Norwegian Red cattle on milk coagulation properties indicated that among all tested single protein genetic variants, only κ-CN genotype affected the acid coagulation properties, whereas polymorphism in β-CN, α_{s1}-CN and β-LG had no significant effect on the firming rate and firmness of the milk acidified with glucono-δ-lactone (GDL). Moreover, the authors also reported a significant effect of the composite genotype of milk caseins on the acid coagulation characteristics, which were the best when the BB-A2A2-AA composite genotype of α_{s1}-β-κ-CN was tested. When the composite genotypes of caseins are taken into account, it should be mentioned than not all combinations of the alleles exist and some are more frequent than others. For example, the AA genotype of κ-CN more frequently coexists with the A2A2 genotype of β-casein, and the composite genotype of β-κ-CN, A2A2-AA is quite a common variant (Gai et al., 2021). Since Ketto et al. (2017, 2019) reported that AA κ-genotype in dairy cattle, frequently co-existing with A2A2 β-CN, contributes to better acid coagulation properties of milk, this may explain better gel properties of A2 yoghurt when compared to its A1 counterpart. Moreover, Hallén et al. (2009) suggested that acid-induced gelation of milk is unaffected by the genotype of any casein fraction, contrary to whey protein β-LG polymorphism and concentration in milk, which improves acid gelation through interaction with casein micelles during heat treatment prior to fermentation. However, the polymorphism of other proteins besides β-CN was not determined in our research. This suggests that the polymorphism of other milk proteins than β-CN should be considered and studied during assessment of A2 milk suitability for the production of fermented dairy products.

In the current study, the better textural and rheological performance of A2 yoghurts compared to A1 yoghurts was confirmed in the sensory evaluation. In the study by

de Vitte et al. (2022), the various β-CN genotypes did not significantly influence milk sensory properties such as appearance, taste or smell. However, in our study, A2 yoghurts were rated higher in sensory testing than A1 yoghurts, attributed to better consistency formed during acid gelation triggered by starter bacteria.

In the study by Ribeiro et al. (2021), milk containing β-casein A1/A2 had better antioxidant activity measured using ABTS method than milk containing only β-casein A2/A2. Additionally, Petrat-Melin et al. (2015) observed that throughout the course of *in vitro* digestion by pepsin and pancreatic enzymes, the A1 and I variants of isolated bovine β-CN showed progressively higher TEAC values (ABTS radical scavenging activity, Trolox-equivalent antioxidant capacity assay) than the A2 variant. However, all intact or digested β-CN variants exhibited similar antioxidant activity against the ABTS radical. The authors suggested that the lower number of His residues in milk of A2 and I β-CN variants, when compared to A1 and B variants (8 vs 9), may contribute to the antioxidant capacity, as previously reported for His, Trp and Tyr single amino acids possessing quenching capacity against ABTS. The lack of differences in the antioxidant capacity between A1 and A2 milk variants, as stated in our study, may be due to the fact that caseins, as well as derived peptides and amino acids, are among many other potentially more potent antioxidant substances present in milk. These include whey proteins, conjugated linoleic acid (CLA), α-tocopherol, β-carotene, coenzyme Q_{10}, phospholipids, vitamins, minerals, trace elements, and milk enzymes, including superoxide dismutase (SOD), catalase (CAT), lactoperoxidase (LPx) and glutathione peroxidase (GSHPx). Additionally, the antioxidant capacity of the fermented milk may be enhanced by the antioxidant activity of lactic acid bacteria used in yoghurt production (Cichosz et al., 2017; Najgebauer-Lejko and Sady, 2015).

On the other hand, the obtained results concern only the finished product and do not exclude the potential antioxidant effects on the human body. For instance, human studies by Deth et al. (2015) suggest an enhanced production of the antioxidant glutathione in plasma following the digestion of A2 milk compared to milk containing both A1 and A2 β-CN variants. Hence, further research on the impact of A2 milk products is therefore justified and needed.

Conclusion

The obtained results indicate that A2 milk is a good raw material for yoghurt production in terms of its acid gelation performance, as well as the textural and sensory properties of the final products. However, further studies are needed to consider the effect of polymorphism of other milk proteins, as well as impact of other factors in this area.

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Received: 12 III 2024
Accepted: 20 VIII 2024