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RESEARCH ARTICLE

Probiotic Minas Frescal cheese added with *Wezmannia coagulans* GBI-30: Proteomic analysis, rheological properties and fatty acid composition

THAIS CRISTINA M SILVA,[1] GUSTAVO LUIS P A RAMOS,[2,*] RAMON SILVA,[1] TATIANA C PIMENTEL,[3] MARIA TEREZINHA S L NETA,[4] SINARA TEIXEIRA,[5] STANISLAU BOGUSZ JUNIOR,[5] ANDRESA CARLA FEIHRMANN,[6] MARCOS RAPHAEL F SILVA,[7] ELIANE T MÁRSICO,[8] MÔNICA Q FREITAS,[8] ERICK A ESMERINO,[8] CÁSSIA P BARROS[7] and ADRIANO G CRUZ[1]

[1]*Departamento de Alimentos, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Instituto Federal de Educação, Rua Senador Furtado, 121, Rio de Janeiro, RJ 20270-021, Brazil,* [2]*Departamento de Bromatologia, Universidade Federal Fluminense (UFF), Rua Doutor Mario Viana, 523, Niterói, RJ 24241002, Brazil,* [3]*Instituto Federal do Paraná (IFPR), Av. José Felipe Tequinha, 1400, Paranavaí, Paraná 87703-536, Brazil,* [4]*Departamento de Tecnologia de Alimentos, Universidade Federal de Sergipe (UFS), Rosa Elze, São Cristóvão, Aracaju, Sergipe 49100-000, Brazil,* [5]*Instituto de Química, Universidade Estadual de São Paulo (USP), Av. Prof. Lineu Prestes, 748, São Carlos, SP 05508-900, Brazil,* [6]*Programa de Pós-Graduação em Ciência de Alimentos, Universidade Estadual de Maringá (UEM), Av. Colombo, 5790, Maringá, PR 87020-900, Brazil,* [7]*Departamento de Quimica, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Instituto Federal de Educação, Rio de Janeiro 20270-021, Brazil, and* [8]*Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Av. Alm. Ary Parreiras, 507, Niterói, RJ 24230-321, Brazil*

*Author for correspondence. E-mail: gustavoanciens@id.uff.br

Background:
Probiotic strains from the Bacillus *genus have attracted significant attention in the functional foods sector thanks to their technological advantages over traditional probiotic strains.*

Aim:
The objective of this study was to examine the impact of adding the spore-forming probiotic Weizmannia coagulans *GBI-30 at various concentrations (6, 8 and 10 log cfu/g) to Minas Frescal cheese.*

Method:
The influence of different probiotic concentrations on the profile of bioactive peptides, fatty acid composition and rheological properties was evaluated.

Major Findings:
The addition of W. coagulans *promoted the release of different bioactive peptides with potential antihypertensive, antimicrobial, antidiabetic and immunomodulatory activities. Moreover, the lipid profile of the probiotic cheeses showed a significant increase in unsaturated fatty acids, monounsaturated (MUFA) and polyunsaturated (PUFA), recognised for their health-promoting effects, including benefits for cardiovascular and metabolic health. These changes likely reflect adaptive responses of the probiotic cells and their impact on the lipid metabolism within the matrix. From a structural standpoint, cheeses supplemented with* W. coagulans *exhibited improved rheological and textural properties, including greater firmness and cohesiveness.*

Scientific and Industrial Implications:
These findings suggest that adding W. coagulans *to Minas Frescal cheese is a promising way to improve the product's quality and provide consumers with a differentiated product that may have positive health benefits. Due to the similar behaviour observed in all* W. coagulans *dosages, and*

taking into account economic reasons, adding 6 log cfu/g is an interesting and adequate option for Minas Frescal cheese.

Keywords Bioactivity, Functional cheese, Probiotic dairy products, Spore-forming probiotics, Textural attributes.

INTRODUCTION

Functional foods have driven the expansion of the probiotic market in recent years, due to their positive role on gut health (Domingos *et al*. 2025). This growth has been fuelled by mounting scientific evidence supporting the health benefits of probiotics, thereby bolstering consumer confidence in these products as natural preventive health solutions. Indeed, the world probiotics market presented USD 5.56 billion in 2023, with a projected compound annual growth rate (CAGR) of 6.9% from 2024 to 2032 (Anonymous 2024).

Probiotics are live microorganisms that confer health benefits to the host when administered in sufficient amounts (Hill *et al*. 2014), with the capacity of remaining viable until they reach the colon, their primary site of action, for clinical benefits to be realised (Hill *et al*. 2014). Therefore, the effective development of probiotic foods requires strategies to ensure the stability and survival of the microorganisms throughout the production chain, the storage and during consumption. Therefore, selecting resistant strains and suitable food matrices is essential to preserve their functionality (Pereira *et al*. 2024).

Recently, probiotic strains from the *Bacillus* genus have attracted significant attention thanks to their technological advantages over traditional probiotic strains belonging to the *Lactobacillus* and *Bifidobacterium* genera (Cinbaş *et al*. 2024). Indeed, spore-forming bacteria, as *Bacillus* strains, present improved resistance to stressful processing technologies with better stability during storage (Cao *et al*. 2020; Almada-Érix *et al*. 2022). Therefore, using naturally more resilient strains is a promising strategy for industrial applications.

Weizmannia coagulans, formerly known as *Bacillus coagulans*, is a nontoxic, nonpathogenic, nonantibiotic-resistant *Bacillus* strain. The European Food Safety Authority (EFSA) considers it is a safe strain, being recognised as Generally Recognized as Safe (GRAS) and Qualified Presumption of Safety (QPS) status (Silva *et al*. 2024). Some research has demonstrated its positive effects in different health disorders (Kang *et al*. 2021; Majeed *et al*. 2023) and nongastrointestinal conditions, such as bacterial vaginosis, obesity and major depression associated with irritable bowel syndrome (Ratna Sudha *et al*. 2012; Majeed *et al*. 2018; Cao *et al*. 2020).

The food matrix plays a critical role in the viability of probiotics (Soares *et al*. 2019; Almada-Érix *et al*. 2021). Fresh cheeses are an excellent delivery vehicle because their intrinsic characteristics support the survival of different probiotic strains, as the high moisture and pH, low sodium content (Sperry *et al*. 2018). Minas Frescal is a typical Brazilian fresh cheese, presenting white colour, soft texture and mild acid flavour (Brazil 2004). In addition, Minas Frescal has widespread acceptance in terms of sensory perception along the Brazilian territory (Rocha *et al*. 2020).

Conversely, the addition of *W. coagulans* in fresh cheese remains limited (Lavrentev *et al*. 2021; Ma *et al*. 2021; Choi *et al*. 2024). A recent study demonstrated this strain presented excellent survival along Minas Frescal cheese shelf life while generating bioactive peptides with a positive effect on the consumer's health (Silva *et al*. 2024). However, its addition can affect its structural properties by changing the peptides profile, the fatty acid composition and the rheological parameters. Therefore, the present study investigated the effect of incorporating different dosages of *W. coagulans* GBI-30 (0, 6, 8 and 10 log cfu/g) on the peptide profile, the fatty acid composition and the rheological properties of Minas Frescal cheese.

MATERIAL AND METHODS

Minas Frescal cheese processing

Minas Frescal cheese was manufactured following the traditional protocol (Silva *et al*. 2024). The total experiment used 10 L of full-fat, pasteurised milk from the same batch (3% fat by weight, Itaocara, Rio de Janeiro, Brazil), divided into similar portions to minimise variability. Each processing used about 3.3 L of milk, with each cheese weighing about 250 g. The milk was then submitted to heating until 35–37°C, being added 40% calcium chloride (30 mL/100 L; Vetec, Rio de Janeiro, Brazil), 85% lactic acid (0.4 mL/L; Macalé) and liquid chymosin (8 mL/L, Ha La, Chr. Hansen, Valinhos, São Paulo, Brazil) were incorporated into the milk with posterior manual stirring with posterior coagulation. After that, the curd was cut into 2-cm cubes and stirred slowly for 5 min. Next, partial whey drainage was performed, followed by the addition of sodium chloride (1% of the milk volume; Cisne, Rio de Janeiro, Brazil) and the addition of *Weizmannia coagulans* GBI-30 (Ganeden Biotech, lyophilised form, 0.67, 6.7 and 670 mg corresponding to 0, 6, 8 and 10 log cfu·g^{-1}, respectively; cheeses Qc, QI, QII and QIII) with additional resting for 30 min. It is important to consider that 67 milligrams of the powder, consisting only of vegetative cells, corresponded to 9 log cfu.g^{-1} of *W. coagulans* GBI-30 spores (Keller *et al*. 2019).

Next, the curd underwent complete whey drainage and moulding at 5°C for 30 min, followed by two 30-min flips. Then, the cheeses were put in polyethylene films and stored at 5°C for 24 h before analyses of the proteomic analysis, rheological assays and fatty acid composition.

W. coagulans exhibits adequate survival during refrigerated storage of Minas Frescal cheese (14 days, 5.26–8.56 log cfu/g), which is consistent with its probiotic status. Additionally, its addition does not affect the composition of the cheeses, with moisture, protein and fat values ranging from 66.3 to 65.9%, 15.3–15.7% and 16.1–16.5%, respectively. In addition, the pH values of the cheeses ranged from 5.95 to 6.05 (Silva *et al*. 2024).

Proteomic analysis

The proteomic analysis was performed according to Scudino *et al*. (2024) using high-resolution, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry using an Autoflex® maX MALDI mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a 355 nm Nd:YAG laser. External calibration was performed using a peptide standard mix. The peptides were uncertificated considering the amino acid sequences of milk proteins [α-, β- and κ-casein, β-lactoglobulin, α-lactalbumin and bovine serum albumin (BSA)]. The Flex Analysis software (version 3.4, Bruker Daltonics) was used for the data processing.

Fatty acid composition

The fatty acid profile was determined using gas chromatography coupled with mass spectrometry (GC–MS; Agilent Technologies, Santa Clara, CA, USA; model 7890A-5975C), considering the previous study Scudino *et al*. (2024). Fatty acid identification and quantification were performed using the Agilent MassHunter Quantitative Analysis software, the results being expressed as a percentage of the total weight of fatty acids.

Considering the fatty acid values obtained, some lipid indexes were computed, as the thrombogenic index and the atherogenic index were calculated (TI and AI, respectively) as proposed by Barłowska *et al*. (2018).

Rheological analysis

Rheological analysis was performed using a TA-XT21 texture analyser (Stable Micro Systems Ltd., Surrey, UK) equipped with a 50 kg load cell and a 35 mm aluminium plate. Cylindrical samples (20 mm in diameter × 24 mm in length) were obtained from the cheeses and individually wrapped in plastic film.

The uniaxial compression test was conducted by compressing the samples to 80% of their initial height at a crosshead speed of 1 mm s^{-1}. The resulting force–displacement data were converted into true stress (σ_t, Eqn 1) and true strain (ε_t, Eqn 2), as described by Gunasekaran and Ak (2003):

$$\sigma_t = \frac{F(t)}{A(t)} = \frac{F(t)L(t)}{A_0 L_0} \quad (1)$$

$$\varepsilon_t = \left|\ln\left(\frac{L(t)}{L_0}\right)\right| = \left|\ln\left(\frac{L_0 - \Delta L}{L_0}\right)\right| \quad (2)$$

where $F(t), A(t), Ao, L(t), Lo$ and ΔL are applied force at time, the cross-sectional area at time, initial cross-sectional area, the sample length at time, the initial sample length and the deformation, respectively.

Stress–strain curves were fitted using a fifth-order polynomial equation (Eqn 3) in according with Ak and Gunasekaran (1992). The following parameters were calculated: modulus of deformability (E_D, Eqn 4), the fracture strain (ε_f) and the work of fracture (W_f, Eqn 5).

$$\sigma_t = \sum_{i=1}^{5} a_i \varepsilon_t^i \quad (3)$$

$$E_D = \left[\frac{d\sigma_t}{d\varepsilon_t}\right]_{\varepsilon_t \to 0} \quad (4)$$

$$W_f = \int_0^{\varepsilon_f} \sigma_t d\varepsilon_t \quad (5)$$

The creep test was conducted by applying a constant force of 0.73 N to the samples for 180 s, during which deformation was measured being the sample recovery was monitored for an additional 180 seconds. The results were expressed as compliance over time, where compliance corresponds to the ratio of strain over time to the applied constant stress ($J(t) = \gamma(t)/\sigma_0$, Eqn 6), being the data fitted to the four-component Burger model using nonlinear regression:

$$J(t) = J_0 + J_1\left(1 - e^{-t/\tau}\right) + \frac{t}{\eta_N} \quad (6)$$

$J(t)$, J_0, J_1, τ and η_N are the compliance over time, instantaneous elastic compliance associated with Maxwell portion, viscoelastic compliance related to Kelvin–Voigt element; retardation time related to Kelvin–Voigt element and Newtonian viscosity associated with Maxwell portion, respectively.

Statistical analysis

The experiment was performed in triplicate, and all analyses were performed at least in triplicate. One-way analysis of variance and Tukey test at a significance level of $P < 0.05$ were used. The XLSTAT 2022.4 software (Addinsoft, Paris, France) was used for the calculations.

RESULTS AND DISCUSSION

Proteomic analysis

Bioactive peptides are protein fragments formed through enzymatic hydrolysis or microbial fermentation. They can modulate biological functions by interacting with specific receptors or enzymes (Wei *et al*. 2022; Saubenova *et al*. 2024), with antimicrobial, antihypertensive, immunomodulatory and other bioactive effects (Akan *et al*. 2025). However, not all peptides exhibit these properties equally (Mohanty *et al*. 2016). Therefore, evaluating the peptide profile of probiotic-enriched Minas Frescal cheese is crucial for understanding how the addition of *W. coagulans* influences the production of bioactive peptides in the cheese.

Peptide profile analysis revealed three peptides with biological activity that were consistently identified across all Minas Frescal cheese samples (Table 1). Notably, among the peptides identified were the angiotensin-converting enzyme (ACE) inhibitory and immunomodulatory activities. ACE-inhibitory peptides are often used to measure antihypertensive activity and are considered a therapeutic approach for treating hypertension. ACE is a metal peptidase that catalyses the transformation of angiotensin I into angiotensin II, a potent vasoconstrictor that has vital importance in blood pressure regulation. Inhibiting ACE leads to vasodilation, which contributes to blood pressure decrease and the prevention of cardiovascular diseases (Lee *et al*. 2023a). In this sense, they can suggest the potential of the biological activity of Minas Frescal cheese added with *W. coagulans* to promote a positive action on cardiovascular and immune health.

The bioactive peptides YQEPVLGPVRGPFPIIV (m/z 1881), LYQEPVLGPVRGPFPIIV (m/z 1994) and LLYQEPVLGPVRGPFPIIV (m/z 2107) all derived from β-casein were consistently detected across all cheese formulations, regardless of probiotic supplementation or concentration. These peptides are well-documented in the literature for their antihypertensive, antimicrobial, antithrombotic and immunomodulatory properties (Coste *et al*. 1992; Yamamoto *et al*. 1994; Bikermo *et al*. 2009; Rojas-Ronquillo *et al*. 2012; Balthazar *et al*. 2024; Scudino *et al*. 2024; Zhang *et al*. 2024). Their consistent presence suggests that they are generated intrinsically from the protein matrix of Minas Frescal cheese. This process is related to the synergistic action of intrinsic milk enzymes and the lactic bacteria which survive along the cheese processing. Under the evaluated conditions, the *W. coagulans* supplementation did not influence the cheese's peptide profile. However, a previous study by our research group found that cheeses supplemented with *W. coagulans* had increased biological activity ($P < 0.05$), especially when the samples presented with higher dosage (Silva *et al*. 2024). This effect was attributed to enhanced proteolysis because this spore-forming probiotic bacteria have proteases which use milk proteins as substrate being able to generate peptides and free amino acids (Keller *et al*. 2019; Cao *et al*. 2022; Zhang *et al*. 2024).

Therefore, although some peptides were identified in all cheeses, the higher bioactivity observed in cheeses added of *W. coagulans* can be a combined action between the cheese matrix and the probiotic culture's enzymatic activity. In this sense, our findings indicate *W. coagulans* has interesting potential as a technological strategy to develop functional dairy foods, in particular, cheeses with enhanced biological properties.

Rheological properties

Figure 1(a) shows the true stress–true strain profiles of the Minas Frescal cheese sample and Table 2 shows the estimated parameters after fitting to a fifth-order polynomial equation.

The control sample exhibited the lowest values, while the probiotic-supplemented samples had a much more prominent profile. The modulus of deformability (ED) reflects a material's resistance to axial deformation and can be interpreted as its stiffness, being obtained from the initial linear segment of the stress–strain curve (Wium and Qvist 1997). The probiotic samples exhibited significantly higher E_D values (43.9–48.4 kPa) than the control sample (32.6 kPa). These results suggest that adding probiotic cultures significantly increases cheese stiffness.

Fracture strain (ε_f) and fracture stress (σ_f) are essential mechanical parameters that offer valuable insights into the structural integrity and mechanical behaviour of cheese prior to rupture. These metrics are related to a variety of cheese properties that significantly impact texture and sensory attributes. Typically, fracture strain presents an opposite

Table 1 Bioactive peptides identified in the Minas Frescal cheese samples by MALDI-TOF-MS analysis.

Protein (origin)	Peptide sequence	m/z	Biological activity	Samples[a]	References
β-casein	YQEPVLGPVRGPFPIIV	1881	ACE inhibitor, antimicrobial, antithrombin, immunomodulator	CONT, QI, QII, QIII	Bikermo *et al*. (2009), Rojas-Ronquillo *et al*. (2012), Balthazar *et al*. (2024)
β-casein	LYQEPVLGPVRGPFPIIV	1994	Immunomodulator	CONT, QI, QII, QIII	Coste *et al*. (1992)
β-casein	LLYQEPVLGPVRGPFPIIV	2107	ACE inhibitor	CONT, QI, QII, QIII	Yamamoto *et al*. (1994), Scudino *et al*. (2024), Zhang *et al*. (2024)

[a]CONT, QI, QII and QIII: control sample, cheese samples with 6, 8 and 10 log cfu g^{-1} of *W. coagulans*, respectively.

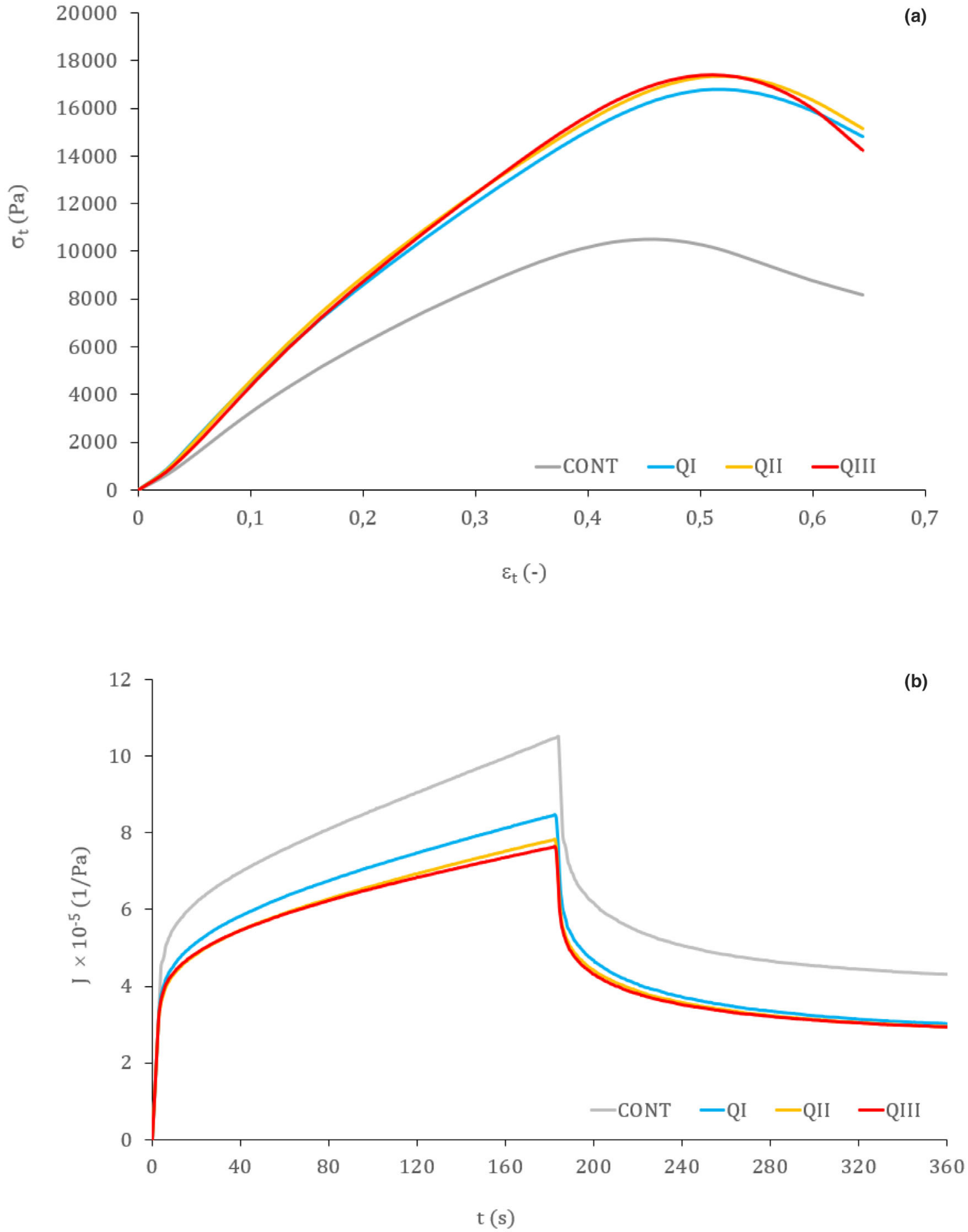

Figure 1 True stress–true strain (σ_t–ε_t, a) and compliance–time (J–t, b) curves of the Minas Frescal cheese samples. CONT, QI, QII and QIII: control sample, cheese samples with 6, 8 and 10 log cfu g^{-1} of *W. coagulans*, respectively.

relation with brittleness, while fracture stress is directly proportional with firmness (Dantas *et al*. 2016). However, this study observed similar behaviour in fracture strain among the cheese samples ($P < 0.05$), suggesting that *W. coagulans* addition had little impact on cheese brittleness. However, the probiotic cheeses presented higher fracture stress values (16.7–17.3 kPa) than the control sample (7.86 kPa), without any differences were observed with respect to probiotic dosage ($P > 0.05$). These results suggest that adding the probiotic culture contributes to firmer cheese that offers greater

Table 2 Rheological parameters of the Minas Frescal cheese samples.

Sample[1]	CONT	QI	QII	QIII
E_D (kPa)	32.6 ± 5.2^{b}	48.4 ± 3.3^{a}	45.8 ± 4.7^{a}	43.9 ± 12.0^{ab}
ε_f (−)	0.61 ± 0.26^{a}	0.53 ± 0.01^{a}	0.53 ± 0.02^{a}	0.51 ± 0.01^{a}
σ_f (kPa)	7.86 ± 5.56^{b}	16.7 ± 0.48^{a}	17.3 ± 1.77^{a}	17.2 ± 1.10^{a}
W_f (kJ/m^3)	3.82 ± 1.14^{b}	5.35 ± 0.27^{a}	5.57 ± 0.82^{a}	5.23 ± 0.26^{a}
$J_0 \times 10^{-7}$ (Pa^{-1})	2.76 ± 0.15^{c}	9.58 ± 0.29^{a}	8.38 ± 0.79^{ab}	7.71 ± 0.99^{b}
$J_1 \times 10^{-5}$ (Pa^{-1})	5.96 ± 0.12^{a}	4.62 ± 0.97^{ab}	4.50 ± 0.95^{b}	4.71 ± 0.56^{ab}
τ (s)	4.06 ± 0.06^{a}	3.32 ± 1.07^{a}	3.57 ± 0.26^{a}	3.65 ± 0.10^{a}
$\eta_N \times 10^6$ (Pa s)	4.00 ± 0.32^{b}	4.42 ± 0.01^{b}	5.48 ± 0.86^{a}	6.04 ± 0.12^{a}

[1]CONT, QI, QII and QIII: control sample, cheese samples with 6, 8 and 10 log cfu g^{-1} of *W. coagulans*, respectively. Different lowercase letters in the same row indicate statistical significance differences (α = 0.05), based on the post hoc Tukey HSD test.

resistance to fracture. Similarly, the work of fracture (W_f), which represents the energy required to rupture the cheese matrix, followed the same trend. The control sample exhibited the lowest W_f value, while the probiotic samples showed significantly higher values. These results suggest that cheeses containing *W. coagulans* require more energy to fracture, indicating enhanced structural integrity.

The observed increase in modulus of deformation, fracture stress and work of fracture in Minas Frescal cheese with *W. coagulans* can be attributed to several factors. These factors include enhanced protein cross-linking, improved texture due to increased enzymatic activity, increased moisture retention, altered microbial metabolites, changes in pH and acidity, strain-specific effects and potential synergy with starter cultures. While these mechanisms can collectively contribute to improvements in cheese texture, their relative significance may have a range, which is related to the probiotic strain and cheesemaking process. Further research is required to evaluate and confirm the exact mechanism in this context.

Figure 1(b) shows the compliance over time ($J(t)$) curves for the Minas Frescal cheese samples. Increased values of compliance were observed in the CONT sample, indicating susceptibility to time-dependent deformation under constant stress. On the reverse, the probiotic cheese samples (QI, QII and QIII) demonstrated lower compliance values, indicating elevated structural resistance to deformation. The findings suggest the addition of *W. coagulans* affects the cheese's viscoelastic behaviour, resulting in a stiffer, more stable matrix. This fact is confirmed by the rheological parameters derived from fitting the data to the four-element Burger model (Table 2), which provides an evaluation of the viscoelastic behaviour of the samples, offering insight into their deformation behaviour and time-dependent mechanical responses during the creep test.

Instantaneous elastic compliance (J_0) represents the material's immediate response to deformation and is inversely proportional to the material rigidity (Dias *et al*. 2022). J_0 values of the probiotic cheese samples were increased when compared to the CONT sample ($7.71–9.58 \times 10^{-7}$ Pa^{-1} against 2.76×10^{-7} Pa^{-1}, $P < 0.05$), suggesting reduced initial stiffness. The viscoelastic compliance parameter (J_1) is defined as the time-dependent deformation under constant stress. The significantly higher (J_1) value in the control sample (5.96×10^{-5} Pa^{-1}) indicates nonprobiotic sample is more viscoelastic and continues to deform over time under sustained stress. In contrast, lower (J_1) values in the probiotic-cultured samples ($4.50–4.71 \times 10^{-5}$ Pa^{-1}) suggesting a somewhat less prone to continuous deformation.

Retardation time (τ) is a measure of how long it takes a material to relax and return to its original shape after experiencing deformation. It offers valuable insights into the time-dependent mechanical behaviour and texture of materials (Zhuang *et al*. 2021). In this study, (τ) values ranged from 3.32 to 4.06 s, with no significant differences among the samples. These moderate values ($1 \leq (\tau) \leq 5$) indicate that all Minas Frescal cheese formulations exhibit a balanced viscoelastic response, capable of adapting to stress without excessively fast or slow relaxation. This mechanical behaviour contributes to the desirable texture of Minas Frescal cheese, combining firmness with a characteristic smoothness.

Newtonian viscosity (η_N) is expresses the material's resistance to flow when subjected to shear forces, reflecting its behaviour under conditions such as spreading or pouring (Rocha *et al*. 2020). Probiotic cheese samples showed higher η_N values when compared to the conventional cheese ($4.42–6.04 \times 10^6$ Pa s against 4.00×10^6 Pa s, respectively, $P > 0.05$). And the increase in η_N was directly proportional to probiotic dosage. This behaviour suggests that the addition of *W. coagulans* leads to cheeses with greater resistance to flow, resulting in reduced spreadability and a firmer, more cohesive texture.

Overall, adding *W. coagulans* to Minas Frescal cheese yields notable rheological and textural benefits. These benefits include developing a firmer texture, which can appeal to

Table 3 Fatty acid profile and health indices of Minas Frescal cheese samples.

Fatty acid (μg/g)	CONT	QI	QII	QIII
Hexanoic acid (C6:0)	71.80 ± 0.02^{c}	79.65 ± 0.02^{c}	93.45 ± 0.01^{b}	114.25 ± 0.02^{a}
Octanoic acid (C8:0)	43.46 ± 0.02^{b}	51.51 ± 0.02^{b}	51.75 ± 0.02^{b}	71.16 ± 0.03^{a}
Decanoic acid (C10:0)	64.98 ± 0.02^{a}	66.09 ± 0.01^{a}	70.98 ± 0.03^{a}	74.45 ± 0.01^{a}
Dodecanoic acid (C12:0)	56.38 ± 0.04^{d}	63.25 ± 0.01^{c}	76.02 ± 0.01^{b}	107.58 ± 0.01^{a}
Miristic acid (C14:0)	201.87 ± 0.04^{a}	203.25 ± 0.01^{a}	206.25 ± 0.01^{a}	211.26 ± 0.02^{a}
Miristoleic acid (C14:1)	29.24 ± 0.01^{a}	34.33 ± 0.02^{a}	36.92 ± 0.02^{a}	39.16 ± 0.02^{a}
Pentadecanoic acid (C15:0)	23.79 ± 0.01^{c}	33.87 ± 0.02^{b}	36.10 ± 0.03^{b}	52.53 ± 0.03^{a}
Palmitic acid (C16:0)	741.22 ± 0.01^{b}	772.27 ± 0.03^{b}	784.50 ± 0.02^{b}	981.70 ± 0.01^{a}
Palmitoleic acid (C16:1)	39.21 ± 0.04^{b}	41.42 ± 0.06^{b}	46.46 ± 0.01^{b}	62.21 ± 0.02^{a}
Heptadecanoic acid (C17:0)	16.62 ± 0.02^{b}	19.26 ± 0.03^{b}	29.29 ± 0.02^{a}	32.04 ± 0.02^{a}
Stearic acid (C18:0)	191.62 ± 0.01^{d}	266.14 ± 0.04^{c}	294.49 ± 0.03^{b}	417.53 ± 0.01^{a}
Oleic acid (C18:1)	557.04 ± 0.02^{d}	634.42 ± 0.06^{c}	656.98 ± 0.01^{b}	688.60 ± 0.05^{a}
9,12-Linolelaidic acid (C18:2n6t)	47.44 ± 0.02^{b}	50.89 ± 0.02^{b}	56.78 ± 0.01^{b}	71.82 ± 0.02^{a}
Linoleic acid (C18:2)	40.66 ± 0.02^{a}	45.51 ± 0.02^{a}	48.36 ± 0.02^{a}	50.06 ± 0.02^{a}
∑ MUFA	711.16 ± 0.74^{d}	749.49 ± 0.55^{c}	835.35 ± 0.50^{b}	942.97 ± 1.05^{a}
∑ PUFA	89.24 ± 0.05^{c}	66.96 ± 0.06^{b}	102.48 ± 0.13^{a}	111.84 ± 0.10^{a}
AI	1.91 ± 0.02^{a}	1.85 ± 0.01^{b}	1.84 ± 0.02^{b}	1.74 ± 0.03^{c}
TI	2.93 ± 0.05^{a}	2.88 ± 0.04^{a}	2.78 ± 0.05^{b}	2.69 ± 0.03^{c}

AI, atherogenic index; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TI, thrombogenic index.
Results are expressed as mean ± standard deviation. Different letters on the same line indicate the presence of a statistical difference ($P < 0.05$) between cheeses. CONT, QI, QII and QIII: control sample, cheese samples with 6, 8 and 10 log cfu g^{-1} of *W. coagulans*, respectively.

consumers seeking a solid, less crumbly consistency. These textural improvements may result from enhanced protein structure and enzymatic activity within the cheese matrix. These enhancements contribute to a better sensory experience and offer opportunities for market differentiation, as consumers increasingly seek products with distinct textures and potential health benefits.

Fatty acid composition

Fatty acid composition, along with the lipid indexes, atherogenic (AI) and thrombogenic (TI) indexes of the Minas Frescal cheese samples (CONT, QI, QII and QIII), is summarised in Table 2. It was observed that the presence of medium-chain fatty acids, including hexanoic (C6:0), octanoic (C8:0), decanoic (C10:0) and dodecanoic (C12:0), as well as long-chain fatty acids such as myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), stearic (C18:0), oleic (C18:1n9c), linolelaidic (C18:2n6t) and linoleic acid (C18:2n6c).

Overall, the cheese samples exhibited intermediate concentrations of medium-chain fatty acids and higher concentrations of long-chain fatty acids, including myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1n9c). These results are consistent with previous studies (Burch *et al*. 2021; Scudino *et al*. 2024) as these fatty acids are common in dairy products and important for evaluating their nutritional quality and health properties. Short- and medium-chain fatty acids play a vital role in regulating cellular metabolism. They also serve as key mediators in intracellular signalling pathways, directly impacting essential physiological and biochemical functions (Gómez-Cortés *et al*. 2018). However, excessive intake of long-chain saturated fatty acids, such as palmitic (C16:0) and stearic (C18:0), is associated with negative aspects of the consumer's health, proportioning higher body fat, elevated inflammatory markers, accentuated risk of insulin resistance and occurrence of type 2 diabetes (Batista *et al*. 2017).

Although the concentrations of various fatty acids were similar in all cheese formulations, the addition of *W. coagulans* altered the lipid profile when compared to the conventional cheese. Indeed, it was noted that the presence of higher levels of medium- and long-chain saturated fatty acids compared with the control sample ($P \leq 0.05$) also improved unsaturated fatty acids, including monounsaturated (MUFA) and polyunsaturated (PUFA) values, this effect being directly proportional to the probiotic dosage, with the highest values observed in QIII, followed by QII and QI ($P < 0.05$). The increased degree of fatty acid unsaturation in probiotic cheeses reflects a universally conserved adaptive response of microorganisms to environmental stress. This shift is commonly associated with the need to maintain membrane fluidity and functionality (Sperry *et al*. 2018). MUFAs, which are naturally present in dairy foods, are associated with a reduced risk of metabolic syndrome, cardiovascular disease and other health-promoting properties (Gómez-Cortés *et al*. 2018). In turn, PUFAs play essential roles in maintaining and promoting health and preventing various chronic diseases. Since the human body cannot synthesise these fatty acids, dietary intake is strongly recommended (Lee *et al*. 2023b).

To better understand the fatty acid composition in parameters reflecting the potential episodes of cardiovascular disease, the atherogenic (AI) and thrombogenic (TI) were calculated. AI values of probiotic cheese samples were lower when compared to the conventional sample (1.74 to 1.85 against 1.91, respectively $P < 0.05$). Regarding the TI, the QI treatment was similar to the control (2.88 and 2.93, respectively; $P > 0.05$), while QII and QIII showed significantly lower values (2.78 and 2.69, respectively; $P < 0.05$). These results suggest that adding *W. coagulans*, particularly at higher concentrations (QII and QIII), could improve the health status of Minas Frescal cheese and reinforce the benefits of adding this probiotic strain to this product (Table 3).

CONCLUSION

Adding *W. coagulans* GBI-30 to Minas Frescal cheese increased the generation of bioactive peptides and resulted in improvement of the fatty acid composition. These findings highlight the inherent potential of fresh cheese as an adequate food matrix to be supplemented with a spore-forming probiotic bacterium.

Additionally, the presence of *W. coagulans* positively affected the cheese's rheological and textural properties, promoting a firmer, more cohesive structure. From technological and sensory perspectives, these improvements may add value to fresh cheeses and align with consumer demand for functional foods that offer health benefits and desirable texture and mouthfeel. Considering all the findings obtained in this study and economic reasons, the dosage of 6 log cfu/g of Weizmannia coagulans GBI-30 in Minas Frescal cheese is the most adequate option. This dosage proportioned similar lipid indexes and the presence of bioactive peptides with different biological activities and resulted in similar rheology parameters.

Further studies are recommended to assess sensory acceptance and validate health-related claims of probiotic Minas Frescal cheese using *in vivo* models or clinical trials.

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AUTHOR CONTRIBUTIONS

Thais Cristina M Silva: Formal analysis; investigation; writing – original draft; visualization; data curation. **Gustavo Luis P A Ramos:** Writing – review and editing; writing – original draft; visualization. **Ramon Silva:** Writing – original draft; formal analysis; visualization. **Tatiana C Pimentel:** Visualization; validation; investigation; writing – original draft. **Maria Terezinha S L Neta:** Validation; visualization; investigation; writing – original draft. **Sinara Teixeira:** Investigation; writing – original draft; visualization; validation. **Stanislau Bogusz Junior:** Visualization; investigation; validation; writing – original draft. **Andresa Carla Feihrmann:** Investigation; visualization; validation; writing – original draft. **Marcos Raphael F Silva:** Investigation; writing – original draft; visualization; validation. **Eliane T Mársico:** Visualization; writing – original draft; investigation. **Mônica Q Freitas:** Visualization; writing – original draft; investigation. **Erick A Esmerino:** Investigation; writing – original draft; visualization. **Cássia P Barros:** Methodology; validation; investigation; formal analysis; supervision; visualization; writing – original draft; software; data curation. **Adriano G Cruz:** Writing – original draft; project administration; resources; supervision; validation; visualization; conceptualization; investigation; methodology; software; data curation; funding acquisition.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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