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Occurrence of *Arcobacter* spp. in Brazilian Minas frescal cheese and raw cow milk and its association with microbiological and physicochemical parameters

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ABSTRACT

Some *Arcobacter* species are considered emerging food-borne pathogens. However, their prevalence in dairy products is not well known. Therefore, the aim of the current research is to know the occurrence of *Arcobacter* species in Minas frescal cheese and raw cow milk and its potential association with physicochemical and microbiological parameters. Two hundred and one food samples (cheese, n = 98 and milk, n = 103) collected between 2016 and 2017 in São Paulo State, Brazil, were analyzed. *Arcobacter* was isolated using an enrichment broth with further filtration over Arcobacter agar. Colonies were characterized by ERIC-PCR, m-PCR and PCR-RFLP and, when was necessary, sequencing of 16S rRNA and rpoB genes were also assessed. Additionally, cheese samples were evaluated in relation to total coliform (TC), thermotolerant coliforms (TtC), coagulase positive staphylococci (CoPS) count and moisture. Raw milk samples were assessed for aerobic mesophilic bacteria count, TC, TtC, Dornic acidity, proteins, fats, lactose, defatted dry extract, total solids and somatic cell counts. The occurrence of *Arcobacter* was 10.2% (10/98) and 16.5% (17/103) in cheese and milk samples, respectively. All isolates were identified as *Arcobacter butzleri*. On the other hand, Multiple Factorial Analysis showed correlation between the presence of this species and the cheese moisture, while this pathogen was correlated with high counts of mesophilic microorganisms and TC in raw milk. No other evaluated parameter in cheese (TC, CoPS) and milk (TtC, Dornic acidity, proteins, fats, lactose, defatted dry extract, total solids and somatic cell counts) showed significant correlation. To date, this is the first report on *A. butzleri* in Minas frescal cheese, therefore its presence in dairy products could be considered a potential public health concern.

1. Introduction

The first published clinical report of *Arcobacter* isolation dates back to the 1970s in samples from pork abortions (Ellis, Neill, O'Brien, Ferguson, & Hanna, 1977). Since then, a significant advance in the taxonomy and epidemiology of this genus has been evidenced by the increase of related publications (Hsu & Lee, 2015).

The genus *Arcobacter* includes some species (*A. butzleri, A. cryaerophilus, A. skirrowii,* and *A. thereius*) considered as emerging human pathogens (Banting & Figueras, 2017; Vandenberg et al., 2004). Those species can trigger several gastrointestinal disorders in humans, such as chronic diarrhea and bacteremia (Ferreira, Júlio, Queiroz, Domingues, & Oleastro, 2014; Webb et al., 2016). *Arcobacter butzleri* is the most clinically significant species for humans (Banting & Figueras, 2017) and it was classified as a serious hazard to human's health by the International Commission on Microbiological Specifications for Foods in 2002 (ICMSF, 2002). *Arcobacter* species have been isolated from different matrices, such as water and foods of animal origin -milk, meat products and shellfish (Collado & Figueras, 2011; Ghaju Shrestha et al., 2017).

In the dairy production chain, *Arcobacter* species have been isolated from different sources, such as cow fecal samples, raw milk and fresh cheese (Merga et al., 2011; Yesilmen, Vural, Erkan, & Yildirim, 2014). There are several possibilities for *Arcobacter* contamination in the dairy industry because the operations in this chain production are quite complex (Giacometti et al., 2015; Serraino & Giacometti, 2014). *Arcobacter* contamination has been investigated in bulk milk tanks (Elmali,

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Can, Elmali, & Can, 2016; Ertas, Dogruer, Gonulalan, Guner, & Ulger, 2010), milking equipment, barn floors, inline filters in milking machinery and, more recently, in cheese (Giacometti et al., 2015; Serraino et al., 2013; Serraino & Giacometti, 2014). However, in comparison with other foods, studies on dairy products are scarce and restricted to a small number of countries (Hsu & Lee, 2015).

The dairy sector is one of the main national social and economic activities in Brazil (Milanez et al., 2018, pp. 45–114), and taking into consideration that Brazil is the 5th largest producer of milk and the largest consumer of milk and dairy products in South America; regarding that information it is justifiable to investigate emerging pathogens in this production chain.

Minas frescal cheese, also known as white or fresh cheese, is one of the most consumed cheeses in Brazil. It is produced in all Brazilian states mainly by small and medium size dairy plants (ABIQ, 2019). Therefore, the main goal of the current research is to determine the occurrence of *Arcobacter* species in retailed Minas frescal cheese and in raw cow milk used for production of dairy products in the Northeast region of São Paulo State, Brazil.

2. Materials and methods

2.1. Sampling

Ninety-eight Minas frescal cheese samples were collected between December/2016 and July/2017: five samples from a cheese processing plant and 93 samples from nine local markets. Additionally, samples of bulk tank milk (n = 103) were collected in 27 dairy farms from different Brazilian cities (Piracicaba, Brotas, São Pedro and Torrinha – São Paulo State), between August and December 2017, in which three samples per farm were collected. Samples were collected under aseptic conditions and transported under cold storage temperature (4 °C) to be analyzed for their microbiological and physicochemical properties within 12 h (milk) and 24 h (cheese).

2.2. Microbiological analysis

Aerobic mesophilic bacteria count, total coliforms (TC), thermotolerant coliforms (TtC) and coagulase-positive staphylococci (CoPS) counts were carried out following the methodology described by Downes and Itō (2001). All analyses were conducted in the Laboratory of *"Higiene e Laticinios"* in the "Escola Superior de Agricultura Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP), Piracicaba-SP, Brazil.

2.3. Physicochemical properties and somatic cell counts

Dornic acidity of raw milk and moisture of Minas frescal cheese were analyzed according to Brazilian normative (Brazil, 2006, p. 141). Milk components (proteins, fats, lactose, defatted dry matter and total solids) and somatic cell counts (SCC) were analyzed in "*Clínica do Leite*" in the "*Departamento de Zootecnia*", ESALQ, USP, Piracicaba-SP, Brazil. The milk composition was estimated by the infrared method – PO ANA 009 and SCC was estimated using flow cytometry (model Bentley Soma count 300, Bentley Instruments Inc., Chaska, MN, USA).

2.4. Arcobacter isolation

For *Arcobacter* isolation, 10 g of cheese or 10 ml of raw cow milk were placed in a sterile sampling bag and homogenized for 1 min in a stomacher with 90 ml of *Arcobacter* Enrichment Broth (AEB) (1.8% peptone, 0.5% NaCl and 0.01% g yeast extract) supplemented with three antibiotics: Cefoperazone (16 mg)-Amphotericin B (10 mg)-Teicoplanin (64 mg) (CAT supplement). Inoculated AEB were incubated under aerobic conditions at 30 °C for 48 h (Shah, Saleha, Murugaiyah, Zunita, & Memon, 2012). After incubation, 0.4 ml of the enriched medium was inoculated on *Arcobacter* broth supplemented with 1.4% agar and CAT using the membrane filtration method. Cellulose acetate membrane filters with a diameter of 47 mm and a pore size of 0.45 μm were used to remove other enteric bacteria (Atabay, Aydin, Houf, Sahin, & Vandamme, 2003). These filters were aseptically removed 1 h after inoculation and the plates were incubated under aerobic conditions for up five days examining the plates every day (Collado, Cleenwerck, Van Trappen, De Vos, & Figueras, 2009a). From each plate in which the bacterial growth was detected, three to five suspected colonies were confirmed by Gram strain and streaked on new plates. Typical *Arcobacter* colonies (2–3 mm in diameter, circular and convex, beige to off-white color) were stored in 15% glycerol at −80 °C.

2.5. Molecular identification and typing

The bacterial DNA was extracted using InstaGene™ DNA Purification Matrix (Bio-Rad, Hercules, CA, USA). Identification at genus level was carried out using the PCR method described by Harmon and Wesley (1996). Positive (*A. butzleri* LMG 10828^T) and negative controls were included in every PCR run.

To eliminate clonal redundant strains in further analyses, the isolates were genotyped with Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR). Only isolates with different genotypes were further identified using the multiplex PCR (m-PCR) method as described by Douidah, De Zutter, Vandamme, and Houf (2010) and by the 16S rRNA gene Restriction Fragment Length Polymorphism (16S rRNA-RFLP) method described by Figueras, Levican, and Collado (2012). Reference strains *A. butzleri* LMG 10828^T, *A. cryaerophilus* LMG 9904^T and *A. skirrowii* LMG 6621^T; CECT 7203 *A. cibarius*, and LMG 24486^T *A. thereius* were included in each analysis.

The 16S rRNA or *rpo*B genes were amplified and sequenced using the universal primer and protocol as described by Harmon and Wesley (1996) and Collado, Cleenwerck, Van Trappen, De Vos, and Figueras (2009b), respectively, for strains that showed inconclusive identification using both identification methods, or when a different RFLP pattern defined for the type strains was obtained. The PCR products were purified using GenElute™ PCR Clean-Up Kit (Sigma-Aldrich), and the amplicons were sequenced bidirectionally using the same primers by an ABI Prism 3500 genetic analyzer (Applied Biosystems, Thermo Fisher Scientific, USA) with the BigDye terminator cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, USA). DNA sequences were compared with the NCBI database using Blast Library to determine the identity of the species.

2.6. Statistical analyses

The association between *A. butzleri* and the Minas frescal cheese characteristics and raw milk was studied using Multiple Factor Analysis (MFA). For cheeses, the microbiological characteristics (TC, TtC and CoPS), physicochemical characteristics (moisture) and the presence of *A. butzleri* were included in the data matrix. For milk samples, microbiological characteristics (TC, TtC and aerobic mesophilic bacteria count), physicochemical characteristics (composition of milk, Dornic acidity), somatic cell count and the presence *A. butzleri* were considered. Statistical analyses were performed using XLSTAT 2018 software for Microsoft Excel® (Microsoft®, WA, USA).

3. Results

The presence of *Arcobacter* was observed in 10.2% (10/98) of Minas frescal cheese samples and in 16.5% (17/103) of raw milk samples (Table 1). The isolates (n = 84) were genotyped by ERIC-PCR, resulting in 12 and 17 different profiles in 29 Minas frescal cheese and 55 raw milk isolates, respectively.

Sixteen isolates needed to be identified by sequencing the 16S rRNA gene, whereas three isolates (Q73C, LC34C, LC96G) were characterized

Table 1
Occurrence of *A. butzleri* isolated from Minas frescal cheese and raw milk.

Samples	N° of identified colonies			Identified species and genotypes	
				A.butzleri	
Type samples	Positives (%)	*Arcobacter* spp	Non-Arcobacter	N° Colonies	N° of ERIC patterns
cheese (n = 98)	10 (10.2)	29	93	15	12
raw milk (n = 103)	17 (16.5)	55	104	21	17
Total (n = 201)	27 (13.4)	84	197	36	29

Table 2
Samples of Minas frescal cheese positive to *A. butzleri*.

Sample code	Total Coliforms MPN/g	Thermotolerant coliforms MPN/g	CoPS CFU/g	Moisture %
Q5	UD	UD	UD	UD
Q27	UD	UD	UD	UD
Q33	UD	UD	UD	UD
Q61	1.6 x10^3	**7.8 x 10^2**	**1.3 x10^6**	88.1 ± 1.53
Q72	8.1 × 10^2	**8.1 x 10^2**	**4.1 x10^7**	76.9 ± 1.05
Q73	2.4 x10^3	24 × 10^2	< 10	85.3 ± 1.36
Q74	1.7 x10	1.7 × 10	< 10	71.9 ± 2.25
Q75	4.5	< 1.8	< 10	80.0 ± 5.21
Q78	2.4 × 10^3	**1.4 x10^3**	**8.9 x10^6**	59.7 ± 1.63
Q79	2.4 × 10^3	**1.0 x10^3**	< 10	69.8 ± 6.61

Note: UD= Undetermined. NMP = most probable number. CoPS: Coagulase-positive staphylococci. **In bold**, samples that are above the limit tolerated by Brazilian legislation (Brazil, 2001).

by sequencing the *rpoB* gene. All isolates from both cheese and milk samples were identified as *A. butzleri*.

3.1. Relationship between microbiological and physicochemical characteristics and the presence of *A. butzleri* in Minas frescal cheese

According to the microbiological analysis performed on the Minas frescal cheese samples, 57.1% (4/7) exceeded 5 × 10^2 CFU/g of TtC (Table 2), which are the maximum limit tolerated by Brazilian legislation. In addition, it was observed that 42.9% (3/7) were greater than 5 × 10^2 CFU/g, which are the maximum limit allowed for CoPS (Brazil, 2001, p. 37). The moisture of the cheeses was higher than 55% when *Arcobacter* was detected.

Fig. 1 shows the representation of dimension 1 and 2 of the MFA run with the physicochemical and microbiological parameters of the Minas frescal cheese samples. The first two dimensions explained up to 55% of the total variance. It was possible to observe that the *A. buztleri* isolates had a slight positive correlation with the moisture content of the cheese (RV = 0.012). However, no correlation was found between the presence of *A. buztleri* and TC, TtC and CoPS (Fig. 1A). The distribution of the samples in which *A. butzleri* was detected are shown in Fig. 1B. A clear pattern is observed: cheese samples negative for *A. butzleri* are located in the 1st dimension, whereas cheese samples positive for *A. butzleri* are distributed in the 1st and 4th quadrants. The MFA analysis was carried out only in 62 out of the 98 Minas frescal cheese samples.

3.2. Relationship of the microbiological and physicochemical analyzes with the presence of *A. butzleri* in raw milk

In Table 3, it is possible to observe that 82.4% (14/17) of the samples of raw milk analyzed positive for *A. butzleri* were above the allowed limits for mesophilic aerobic microorganisms (3.0 x10^5 CFU/ml) (Brazil, 2011, p. 24). Similarly, 41.2% (7/17) of samples contaminated with *A. butzleri* exceeded the 5.0 × 10^5 cells/ml-which represents the maximum limit allowed for SCC. Regarding the milk components (proteins, fats, lactose, defatted dry extract and total solids) were within the parameters required by the same regulation (Brazil, 2011, p. 24).

MFA was performed using the physicochemical and microbiological parameters of the raw milk samples, and results are shown in Fig. 2. Overall, MFA explained 51.43% of the total variance using the first 2 dimensions. The presence of *A. butzleri* was correlated (RV = 0.12) with the aerobic mesophilic counts and TC, which means that the samples positive for *A. butzleri* showed high counts of aerobic mesophilic and TC (Fig. 2A). However, no correlation was found between *A. butzleri* and TtC. Moreover, no correlation was observed between *A. butzleri* and SCC, chemical composition and Dornic acidity. It was possible to observe the correlation between the SCC and the milk composition (RV = 0.22), which is already known and widely reported (Lindmark-Månsson, Bränning, Aldén, & Paulsson, 2006; Malek dos Reis, Barreiro, Mestieri, Porcionato, & dos Santos, 2013). In Fig. 2A, the position (dimensions 1 and 2) of the raw milk positive for *A. butzleri* is shown. In the 1st and 4th quadrants, the negative samples for *A. butzleri* are grouped, implying they present similar characteristics in terms of physicochemical parameters. On the other hand, positive samples for *A. butzleri* are spread over the 2nd and 3rd quadrants. To facilitate the presentation of the results in Figs. 1B and 2B, positive samples for *A. butzleri* are highlighted and within an ellipse.

4. Discussion

4.1. *A. butzleri* on Minas frescal cheese and raw milk

Research has shown that *A. butzleri* followed by *A. cryaerophilus* and *A. skirrowii* are the most frequently species isolated from milk and dairy products (Giacometti et al., 2013; Serraino & Giacometti, 2014; Yesilmen et al., 2014). These data agree with those found in the present investigation (Table 1), considering that all isolates obtained from Minas frescal cheese and raw milk were identified as *A. butzleri*.

To date, this is the first report of *A. butzleri* isolated from Minas frescal cheese in São Paulo, Brazil. This product is a fresh cheese with a high moisture content (> 55%) and is consumed as a ready to eat snack, and for the preparation of sandwich, salads, and other dishes. The current legislation regarding the manufacture of Minas frescal cheese in Brazil requires the pasteurization of milk to avoid the presence of pathogens (Brazil, 2001, p. 37). However, as shown in Table 1, it was possible to isolate *Arcobacter* in 10.2% of the cheese samples. Yesilmen et al., (2014) isolated *Arcobacter* in 56% (28/50) from fresh village cheese produced with raw milk in Turkey. All analyzed cheeses had sanitary inspection stamp on the package. Thus, it is assumed that these samples were produced with pasteurized milk.

Giacometti et al. (2014) did not find *Arcobacter* in cheese samples produced with pasteurized milk in Italy. Based on these observations, we may infer that the milk used to manufacture the cheese samples studied herein probably did not undergo an adequate heat treatment or a post-pasteurization contamination occurred (Hilton, Mackey, Hargreaves, & Forsythe, 2001).

All Minas frescal cheese samples positive for *A. butzleri* had a moisture content higher than 55% (Table 2). The high moisture allied

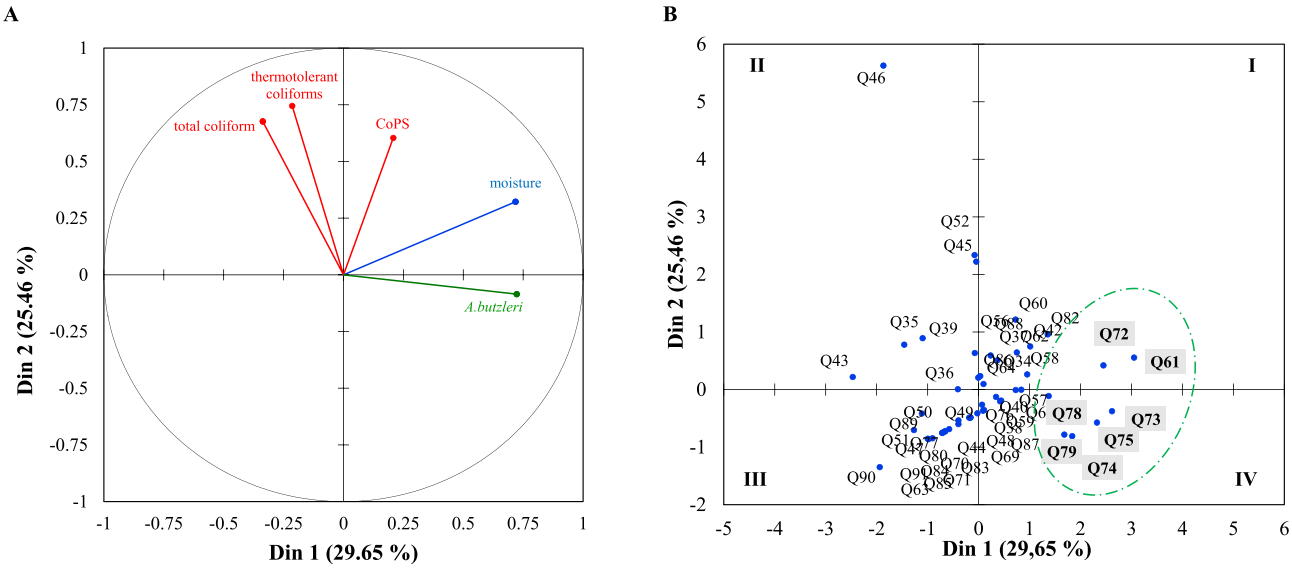

Fig. 1. Multiple factor analysis (MFA) of Minas frescal cheese samples for dimensions 1 and 2. **(A)** Red lines represent the microbiological analyses, blue line represents the physical analyses and green line represent the positive *A. butzleri* samples. **(B)** Position of positive samples for *A.butzleri*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) Note: CoPS = Coagulase-positive staphylococci.

to the availability of nutrients in cheeses can promote a suitable environment for survival and growth of *A. butzleri*. In fact, Giacometti et al., (2015) observed the growth of *A. butzleri* and *A. cryaerophilus* in a similar type of fresh cheese (ricotta), showing that at 6 °C the bacterial population remained stable; on the other hand, the bacterial population increased when cheeses were stored at 12 °C.

Additionally, by means of the MFA, it was not possible to find a correlation between the presence of *A. butzleri* and TC, TtC and CopS in the cheese samples analyzed. However, these results should be interpreted considering the low number of samples analyzed, implying that further studies with a larger sample set should be conducted to better elucidate the correlation between the presence of *A. butzleri* in the Minas frescal cheese and other physicochemical and microbiological factors.

High counts of TC, TtC and CopS indicates process failures, such as cross contamination by inadequate flow in the processing cheese plants, insufficient time and temperatures of pasteurization, poor hygiene of the manipulators, abuse of storage temperature (Tortorello, 2003). Failures in hygiene procedures and microbial biofilms can also be a cause of post-heat treatment contamination (Marchand et al., 2012). Regarding the raw milk samples, 16.5% (16/103) were positive for *A. butzleri*, which is above the prevalence reported by Ertas et al., (2010) and Revez, Huuskonen, Ruusunen, Lindström, and Hänninen (2013) in Turkey and Finland, respectively. The source of contamination of cheese is diverse. Milk is usually contaminated with microbial pathogens because of endogenous contamination – mastitis or from exogenous sources, such as the contamination during or after milking (e.g., feces contamination on the skin, udder/tits, environment) (Verraes et al., 2015).

The presence of *Arcobacter* in raw milk has been widely reported in multiple publications around the world (Shah et al., 2012; Van den Abeele, Vogelaers, Van Hende, & Houf, 2014). An investigation carried out by Logan, Neill, and Mackie (1982) indicates that *A. cryaerophilus* could be a causative agent of mastitis; however, this hypothesis has not yet been proven. The only previous Brazilian report of *Arcobacter* in milk samples was made by Pianta, Passos, Hepp, and Oliveira (2007),

Table 3
Samples of raw milk positive to *A. butzleri*.

Sample code	Dornic acidity	Total Coliforms MPN/ml	Thermotolerant coliforms MPN/ml	Aerobic mesophilic bacteria count CFU/ml	fat (% m/m)	Protein (% m/m)	Lactose (% m/m)	ST (% m/m)	Solids- not-fat (% m/m)	SCC/ml
L22	16.0 ± 0.00	> 1.1×10^4	1.4×10	$1.8 \times 10^5 \pm 0.51$	3.75	3.40	4.36	12.54	8.79	**$5.2\ x10^5$**
L23	16.0 ± 0.47	> 1.1×10^4	< 3	**$7.2x10^6 \pm 1.20$**	2.06	3.16	4.52	10.71	8.65	$8.5\ x10^4$
L24	13.0 ± 0.81	9.2×10	2.3×10	$7.3 \times 10^3 \pm 2.33$	3.35	3.07	4.65	12.07	8.72	$2.4\ x10^5$
L27	15.0 ± 0.00	> 1.1×10^4	2.3×10	**$5.3x10^6 \pm 0.61$**	3.81	3.28	4.55	12.64	8.83	**$1.1\ x10^6$**
L28	15.0 ± 0.47	> 1.1×10^4	3.6	**$2.5x10^6 \pm 0.82$**	3.02	3.09	4.71	11.80	8.78	**$5.3\ x10^5$**
L29	14.5 ± 0.23	> 1.1×10^4	< 3	**$2.8x10^6 \pm 0.54$**	2.91	2.69	4.10	10.69	7.78	$2.2\ x10^5$
L34	15.0 ± 0.00	4.30×10^2	< 3	**$6.6\ x10^5 \pm 0.39$**	3.95	3.17	4.35	12.51	8.56	**$5.4\ x10^5$**
L40	16.0 ± 0.00	> 1.1×10^4	3.6	**$6.3x10^7 \pm 1.34$**	4.83	3.70	4.01	13.62	8.79	**$1.5\ x10^6$**
L46	16.0 ± 0.00	1.1×10^4	< 3	**$7.3x10^5 \pm 0.91$**	3.10	3.34	4.55	12.00	8.90	$5.3\ x10^4$
L47	16.0 ± 0.00	4.3×10	4.3×10	$1.2 \times 10^5 \pm 1.33$	3.02	3.20	4.70	11.90	8.88	$4.6\ x10^4$
L48	17.0 ± 0.47	> 1.1×10^3	< 3	**$4.5x\ 10^5 \pm 0.22$**	3.33	3.26	4.72	12.28	8.95	$7.7\ x10^4$
L63	14.0 ± 0.23	1.1×10^3	3.6	**$3.6x\ 10^5 \pm 0.40$**	3.43	2.96	4.35	11.72	8.29	**$5.4\ x10^5$**
L70	15.0 ± 0.00	> 1.1×10^3	3.6	**$1.4x\ 10^6 \pm 0.27$**	2.70	3.31	4.44	11.44	8.74	$1.7\ x10^5$
L79	14.0 ± 0.23	3.8×10^2	3.6	**$1.3x10^7 \pm 1.69$**	3.10	2.73	4.00	10.89	7.79	$2.8\ x10^5$
L82	17.0 ± 0.47	2.4×10^3	< 3	**$1.5x10^7 \pm 0.71$**	2.82	3.15	4.59	11.50	8.68	$2.6\ x10^5$
L96	11.5 ± 0.47	2.4×10^3	2.4×10^2	**$3.1x10^7 \pm 0.54$**	1.98	2.49	3.23	8.86	6.88	$5.0\ x10^5$
L101	15.5 ± 0.23	1.1×10^3	9.2	**$2.8x10^6 \pm 0.92$**	3.51	3.11	4.36	12.01	8.50	**$5.5\ x10^5$**

Note: NMP = Most Probable Number, CFU = Colony Forming Unit, ST = , SCC = Somatic Cell Count. **In bold**, samples that are above the limit tolerated by Brazilian legislation (Brazil, 2011).

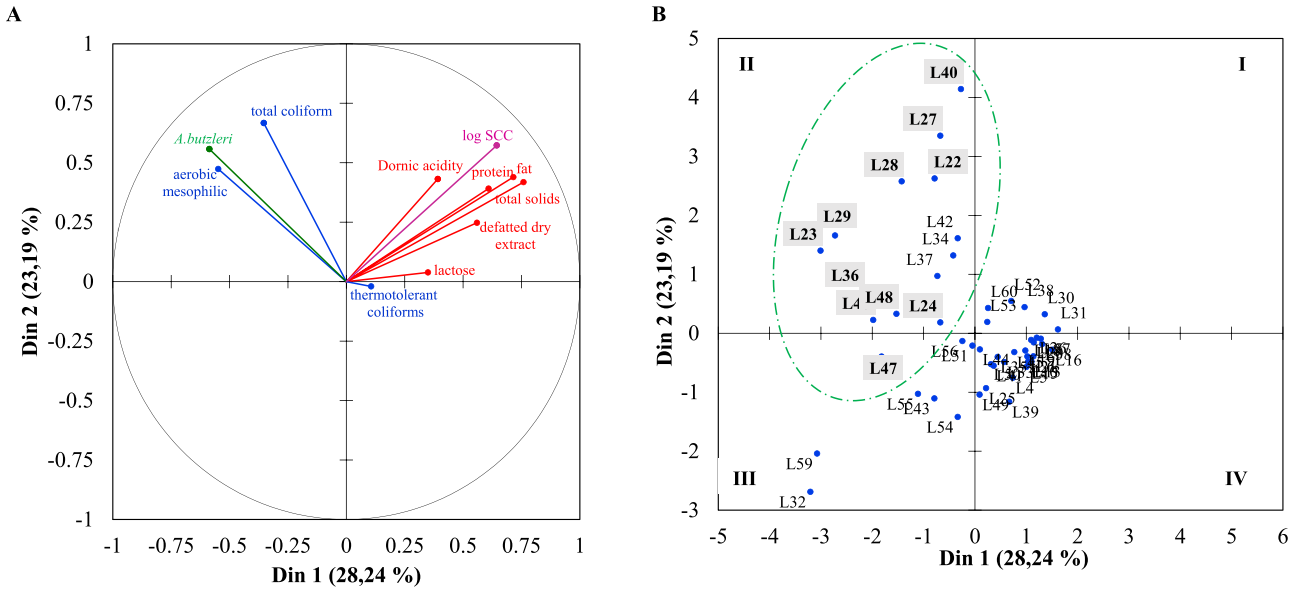

Fig. 2. Multiple factor analysis (MFA) of raw milk samples for dimensions 1 and 2. **(A)** Red lines represent the physicochemical analyses, blue lines represent the microbiological analyses, green line represent samples positive for *A. butzleri* and purple line represent somatic cell count. **(B)** Position of positive samples for *A. butzleri*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) Note: SCC = somatic cell count.

who obtained a lower prevalence (3.2%) compared to the data obtained in the current research. However, it is important to consider that those authors analyzed milk from subclinical mastitis cows.

Multivariate statistical analysis has been used to correlate data from different natures. By using MFA, it is possible to find a correlation between the presence of *A. butzleri* with the TC and the mesophilic count in the raw milk analyzed. Our results suggest that the presence of *A. butzleri* could have an environmental origin. The bacterium adapts to environmental conditions and its niche can be barnyard animals (Merga et al., 2011, 2013).

In fact, *A. butzleri* is quite common in cow feces (Kabeya et al., 2003; Shah et al., 2013; Vilar et al., 2010). Their prevalence ranged from 4 to around 40%, depending on the methodology used for detection (Golla et al., 2002; Grove-White, Leatherbarrow, Cripps, Diggle, & French, 2014; Vilar et al., 2010). Technical errors in the milking process, precarious hygiene conditions, and other factors may increase the risk of milk contamination by bacteria from environmental or fecal origin. However, in the present investigation, no correlation was found between the presence of *A. butzleri* and TtC, which could be a clear evidence of fecal contamination. Nevertheless, the correlation between *A. butzleri* with total coliforms (Fig. 2) could lead to reinforce that the presence of *A. butzleri* is caused by environmental conditions.

However, in other environments, such as water, seawater and sewage, the correlation between *Arcobacter* and high counts of total coliforms was already reported (Collado, Inza, Guarro, & Figueras, 2008). Similarly, Lee, Agidi, Marion, and Lee (2012) observed a significant correlation between *Arcobacter* and the human-specific fecal marker, *HuBAc*, on the beaches of the lake Erie located in Ohio, USA. In foods, Salas-Massó, Figueras, Andree, and Furones (2018) indicated that the presence of *E. coli* can predict the presence of pathogenic *Arcobacter* species in shellfish samples harvested from water with temperatures lower than 26.2 °C. It is important to clarify, that this phenomenon has not been well elucidated because of the complexity of bacterial communities and due to the limited use of specific bacterial groups as indicators of *Arcobacter* species (Hsu, Mitsch, Martin, & Lee, 2017; Leight, Crump, & Hood, 2018).

The quality of milk is always associated with its physicochemical composition as well as its microbiological quality (Oliver, Jayarao, & Almeida, 2005). As shown in Table 3, milks that were positive for *A. butzleri* presented high somatic cell counts (> 5.0 x10^5 somatic cells/ml), exceeding the limits of 10^5 somatic cells/ml, thus indicating contaminated udders (Lindmark-Månsson et al., 2006). By analyzing MFA (Fig. 2A), the factor SCC did not show a correlation with the presence of *A. butzleri*, which reinforces the hypothesis that the presence of *A. butzleri* in raw milk could have an environmental origin.

As already stated by other authors (Hamann & Kromker, 1997; Schukken, Wilson, Welcome, Garrison-Tikofsky, & Gonzalez, 2003), the somatic cells count always have a correlation with the chemical composition of the milk, which is observed in Fig. 2A. In relation to Dornic acidity, which is also an important indicator of microbiological quality (Gargouri, Hamed, & Elfeki, 2013), no correlation was found with *A. butzleri*.

To guarantee microbiological quality, milk should be maintained at refrigerated temperatures after proper pasteurization (USDA, 2019). The current Brazilian regulation (Brazil, 2011, p. 24) stipulates that the raw milk tanks should be kept at 4–7 °C in dairy farms, to avoid the multiplication of pathogenic microorganisms. Giacometti et al., (2014) observed that when UHT milk was stored at 6 °C, the *A. butzleri* remained stable, whereas when the storage temperature was increased to 20 °C, *A. butzleri* counts increased considerably.

5. Conclusion

This work shows the occurrence of *A. butzleri* in Minas frescal cheese and raw milk in the state of São Paulo, Brazil. This is the first published report on *A. butzleri* isolation in Brazilian frescal cheese. The occurrence of *A. butzleri* can be a public health problem considering that it is a ready to eat product. It is important to highlight that infectious dose and the behavior of this bacterium in food are not yet well comprehended. Additionally, the results of the present investigation point out the need to control *A. butzleri* from the post-milking period. A continuous microbiological control of milk should be carried out in the whole chain of production and distribution of the Minas frescal cheese.

Declarations of interest

None.

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