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Assessment of physicochemical, textural and microbiological properties of brazilian white mold surface-ripened cheeses: a technological approach

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ABSTRACT: There are no specific technical regulations regarding the identity and quality of white mold surface-ripened cheeses in Brazil. These cheeses are sold both whole (Camembert-type) and in wedges (Brie-type). The aim of the study was to evaluate the physical and chemical properties; technological parameters and microbiological safety of 20 whole cheeses (Camembert-type) and 16 cheese wedges (Brie-type) produced in Brazil. Samples showed a wide range in sodium (91.0-731.0 mg/100 g, cheeses wedges) and calcium (238.0-1100.0 mg/100g, whole cheeses) contents. The cheese groups presented no significant differences in relation to the majority of the analyzed parameters. Listeria monocytogenes was reported in 5% of the whole cheese samples. The other microbiological parameters were in accordance with the current legislation, RDC nº 12/2001 of Anvisa. The comparative assessments between these two cheeses indicated that they are different. In addition, the wide range of results indicated a lack of processing standardization. The mean values of the physicochemical and textural parameters should be considered as recommended for these cheeses in Brazil.

Key words: cheese quality, chemometrics, Camembert-type cheese, Brie-type cheese.

Avaliação das propriedades físico-químicas, de textura e microbiológicas de queijos brasileiros maturados com mofo branco: uma abordagem tecnológica

RESUMO: No Brasil, não há uma regulamentação técnica específica sobre a identidade e qualidade de queijos maturados com mofo branco na superficie. Estes queijos são comercializados inteiros (tipo Camembert) e em cunhas (tipo Brie). O objetivo da pesquisa foi avaliar as propriedades físicas e químicas; parâmetros tecnológicos e marcadores microbiológicos de 20 queijos inteiros (tipo Camembert) e 16 cunhas de queijo (tipo Brie) produzidos no Brasil. Os teores de sódio e cálcio apresentaram elevada amplitude (8,0 e 4,6 vezes o valor mínimo, respectivamente). Os valores médios destes queijos não apresentaram diferenças significativas em relação à maioria dos parâmetros analisados. Listeria monocytogenes foi encontrada em 5% das amostras de queijo tipo Camembert. Os demais parâmetros microbiológicos estavam de acordo com a legislação vigente, RDC nº 12/2001 da Anvisa. Esta avaliação comparativa entre estes dois queijos indica que são diferentes. Além disso, os valores com elevada amplitude correspondem a falta de padronização do processamento. Os valores médios poderiam ser considerados como recomendados para estes queijos no Brasil.

Palavras-chave: qualidade de queijo, quimiometria, queijo tipo-Camembert, queijo tipo-Brie.

INTRODUCTION

The production of white mold surfaceripened cheeses in Brazil started around the 1930s in the state of Minas Gerais, which is considered to be the first time that this class of cheese was produced in the southern hemisphere. In 2017 the production of these cheeses in Brazil was 3,289 tons (72% and 28% of Brie and Camembert-type, respectively), which was produced by fourteen companies (BRASIL, 2018). These cheeses are frequently consumed at home or in restaurants in colder regions of southern Brazil, with friends or at social gatherings, and usually with wine (JUDACEWSKI et al., 2019). The largest producer of this class of cheese worldwide is France, with 263,000 tons made from bovine milk. It is estimated that this class of cheese represents 2-3% of cheese production worldwide (SPINNLER, 2017).

These cheeses are coated with *Penicillium* camemberti or *P. candidum* mycelium and they have

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a velvety white surface. This mold is responsible for promoting the characteristic appearance, taste and aroma of the cheese (BATTY el al., 2019; SPINNLER, 2017). Brazilian white mold surfaceripened cheeses have significant differences when compared to the French original versions of such cheeses. These differences include the following: (1) Brazilian cheese is produced using milk from different breeds of dairy cows (mainly Holstein and Jersey) with distinct animal feeding and climatic conditions; (2) commercialization occurs at 2-3 weeks of ripening (fresh to partially ripened) due to the low sensorial perception of bitterness and residual ammonia (factors which are unpalatable for the majority of Brazilian consumers) at that stage; (3) Brazilian cheese may contain less moisture and be harder in order to maintain its structure in the higher temperatures of a tropical climate; and (4) there is no specific Brazilian legislation regarding the quality standards for white mold surface-ripened cheeses (LECLERCQ-PERLAT, 2011; FRANCE, 2013; GALLI et al., 2016; JUDACEWSKI et al., 2016, FRANCE, 2018).

Brazilian cheeses with white mold on the surface were not previously characterized with respect to their physical, chemical and microbiological attributes. This information could be used directly by the cheese-making industry in order to establish processing standards, and by inspection authorities to establish quality and identity standards. Thus, the aim of the present study was to evaluate the physical and chemical properties, texture profile and microbiological safety of white mold surface-ripened cheeses produced in Brazil.

MATERIALS AND METHODS

Materials

Samples of whole (n=20, Camembert-type cheeses) and wedges (n=16, Brie-type cheeses) of white mold surface-ripened cheeses were collected in different regions of Brazil. These samples represent 80% of this class of cheese produced in the country. All the samples were produced by establishments inspected by the Federal Inspection Service (SIF) regulated by the Ministry of Agriculture, Livestock and Food Supply (MAPA). The cheese samples were collected by MAPA inspectors and forwarded by refrigerated mail to the State University of Ponta Grossa (UEPG, Paraná, Brazil) and were subsequently refrigerated (5 to 7 °C) until further analysis (BRASIL, 2017).

Methods

Label specifications, dimensions and analytical procedure

The weight (g), diameter (mm) and height (mm) of all the samples were determined. The price of the cheese per kg was expressed in US dollars. The colorimetric analysis and texture profile were then analyzed. The samples were subsequently crushed in a food processor (Philips Walita, model RI 7620, Brazil) homogenized and analyzed for their physicochemical and microbiological parameters. All the analyses were performed in triplicate.

Color measurement

The instrumental color of the samples was analyzed by reflectance using a MiniScan EZ colorimeter (HunterLab, Reston, Virginia, USA). The cheese samples were divided into four quadrants, including the two surfaces (top and bottom). The L^* , a^* and b^* values were recorded. The whiteness Index (WI) of the surface of the cheeses was calculated according to JUDACEWSKI et al. (2016).

Texture profile analysis (TPA)

The TPA was performed instrumentally with a TA.XTplus texture analyzer (Stable Micro Systems® texturometer, Godalming, UK) with a 5,000 g load cell. The cheeses were cut into cylinders with 20 mm diameter and 20 mm height (JASTER et al., 2019). The test speed was 0.8 mm/s with dual compression, which was 30% of the initial height of the sample, using an aluminum cylindrical probe of 36 mm diameter, at 25 °C, in accordance with MARINHO et al. (2015). The parameters of hardness (N), cohesiveness, chewiness (N.s) and resilience were analyzed using Exponent Lite 6.1.4.0 software for Windows. The analyses were carried out in six replicates per sample.

Physicochemical analysis

The determination of moisture was carried out gravimetrically after drying the samples in an oven at 105 °C up to constant weight. The determination of pH (pH 21 meter, Hanna, Cotia, Brazil), acidity, lactose, lipid content and total nitrogen (Kjeldahl method), converted to protein with a conversion factor of 6.38, were made according to the AOAC (2016). The dry extract was determined by the difference in moisture content subtracted from 100, and the non-fat dry extract (NDE) was determined by the difference between the dry extract and lipids content. The ash content was determined by incineration in a muffle furnace (2310, Fornos Jung Ltda, Blumenau, Brazil)

at 550 °C for 6 h (IAL, 2008). The sodium content was determined using the method of Volhard modified by MARGOLIES & BARBANO (2018). The calcium level was evaluated by complexometric titration with EDTA (IAL, 2008).

Microbiological analysis

A quantity of 25 g of cheese was mixed with 225 mL of 2% sterile peptone water and then homogenized in a stomacher (Stomacher Homogenizer 130, Ethik technology, São Paulo, Brazil) for one minute. Decimal dilutions were made, followed by plating in duplicate for each dilution. Listeria monocytogenes and Salmonella spp. were studied using the conventional procedures described by WEHR & FRANK (2004); results were expressed by the presence or absence of the pathogens in 25 g of sample. The coagulase-positive Staphylococcus (CPS) were enumerated using Baird-Parker agar (Oxoid), incubated at 35 °C for 48 h, followed by catalase, coagulase, and thermonuclease tests of typical and atypical colonies. The most probable number (MPN) of thermotolerant coliforms at 45 °C was determined following the methodology of the American Public Health Association (WEHR & FRANK, 2004).

Statistical analysis

The experimental data were presented as mean ± standard deviation (SD) and as minimum and maximum values. Firstly, the samples had their normality checked (Shapiro-Wilk's test), and the differences between the samples associated in groups (whole and cheeses wedges) were assessed using Student's t-test. The data were also submitted to principal component analysis (PCA) in order to explore their structure and to look for associations between the variables and the samples. For this purpose, all the variables were firstly auto scaled to standardize the statistical importance of all the responses. A 2D scatter plot was then used to distinguish the differences between the samples obtained by the PCA results. All the statistical analyses were performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

RESULTS AND DISCUSSION

The samples were divided into whole cheeses (Camembert-type) and cheese wedges (Brietype). These two types of cheese currently have values between US\$ 16-27.0/kg (Table 1) and a short ripening period (10-21 days).

The dimensions of the samples of both cheeses presented significant difference (p<0.05) and high variability (33 and 51% for the height and weight of whole cheeses, respectively). The whole cheese presented a cylindrical shape with mean weight (190 g), diameter (8.9 cm) and height (3.2 cm). The wedges of cheese weighed 60-178 g and mean diameter (12.5 cm) and mean weight (750 g) of the cheeses presented high variability (Table 1).

The moisture content for both cheeses ranged from medium (36-45.9%) to high (46-54.9%) (Table 1). These values were lower than those reported by LECLERCQ-PERLAT (2011) for French white mold cheeses (50-60%). The causes of these variations and the lower moisture content in relation to French cheeses may be due to different humidity and temperatures used in the ripening process; lack of efficient control of humidity and design of maturation chambers (LECLERCQ-PERLAT et al., 2015; JUDACEWSKI et al., 2016).

The pH values showed significant difference (p<0.05). The values for the fresh cheeses were < 5.5 and for well-ripened cheeses they were > 6.5 (ABRAHAM et al., 2007). The variations in the lactose and acidity content confirmed this information (Table 1). The pH of bovine milk is 6.6-6.8 and with lactic fermentation it decreases to 4.6 and 4.8 (when taken from the mold). *P. candidum* consumes lactic acid for growth, de-acidifying the surface of the cheese and promoting a migration of lactate from the interior to the exterior of the cheese (ABRAHAM et al., 2007). Thus, the external pH (rind) can be around 7.0 and the internal (mass) around 6.0 at the end of the ripening period (5-6 weeks) (SPINNLER, 2017).

All the samples of both cheeses were classified as soft (moisture on a fat-free basis (MFFB) > 67%) and high-fat (fat on a dry basis (FDB) > 60%) (BYLUND, 2015), which are typical features of Camembert-type and Brie-type cheeses (LECLERCQ-PERLAT, 2011). The high lipid (> 60 g/100 g) content may have been due to the addition of cream to the milk. In some cases, lipids are added in the processing, modifying not only the lipid content in the cheese, but also attributing sensorial characteristics such as flavor, aroma and texture (PHADUNGATH, 2005). These cheeses with added lipids have less syneresis and melt at higher ambient temperatures (>20 °C).

The minimum, average and maximum protein contents for both cheeses were similar (Table 1). The protein contents showed a high variability (13.0-22.5 g/100 g). Similar results (13.71-19.76 g/100 g) were reported by VOIGT et al. (2011) in Camembert-

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Table 1 - Characteristics of white mold surface-ripened cheeses produced in Brazil.

Analytic Parameters	Whole cheeses (n=20)	Cheese wedges (n=16)	p
Height (mm)	32.7 ± 3.6 (26.0-39.3)	29.5 ± 2.5 (26.0-35.0)	0.01
Diameter* (mm)	88.7 ± 13.1 (67.0-101.0)	125.2 ± 29.7 (80.0-176.0)	0.01
Price/kg **(US\$)	18.9 ± 3.1 (16.3-26.9)	19.5 ± 2.5 (17.0-27.6)	0.47
Moisture (g/100g)	47.5 ± 3.6 (39.7-54.5)	47.9± 4.6 (39.6-54.8)	0.84
pH	5.6 ± 0.7 (4.6-7.3)	6.4± 0.6 (5.4-7.1)	0.03
Acidity (g/100g)	0.5 ± 0.2 (0.1-0.9)	0.5±0.1 (0.2-0.8)	0.74
Lipids (g/100g)	57.9 ± 4.7 (48.1-67.1)	56.1± 4.4 (44.1-62.5)	0.22
Protein (g/100g)	18.1 ± 2.7 (13.2-21.6)	19.0 ± 3.1 (12.8-22.5)	0.40
Lactose (g/100g)	2.9 ± 2.0 (0.0-7.5)	4.4 ± 2.9 (0.4-9.0)	0.08
NDE (g/100g)	22.1 ± 3.2 (18.2-31.1)	23.2 ± 4.0 (18.0-32.9)	0.31
Dry extract (g/100g)	52.4 ± 3.6 (45.5-60.3)	52.1± 4.6 (45.2-60.3)	0.84
Ash (g/100g)	3.6 ± 0.5 (2.8-4.7)	3.4 ± 0.7 (2.6-4.9)	0.37
Sodium (mg/100g)	462.6 ± 204.4 (111.0-645.8)	472.2± 180.6 (91.0-731.0)	0.88
Calcium (mg/100g)	539.7 ± 275.4 (238.0-1100.0)	493.5 ± 176.2 (280.6-842.3)	0.59
WI	90.9 ± 1.9 (87.1-93.8)	69.9± 3.9 (60.2-76.0)	0.01
Hardness (N)	$6.1\pm 1.0 \\ (2.9-8.1)$	5.8 ± 0.9 (3.8-7.4)	0.31
Cohesiveness	0.5 ± 0.1 $(0.2-0.7)$	0.7 ± 0.9 (0.3-3.9)	0.34
Chewiness (N.s)	1.8±2.1 (0.07-8.0)	1.5±1.6 (0.1-5.9)	0.98
Resilience	0.2 ± 0.1 $(0.1-0.3)$	0.2 ± 0.3 (0.1-1.3)	0.32

Note: *diameter of the cheese based on the size of cheese wedge (radius x 2); ***R\$ 1.00 = US\$ 0.44; NDE = non-fat dry extract; lipids and lactose contents were determined in wet basis; WI = whiteness index.

type cheese. This variability may have been due to factors such as the protein content in the milk, the processing technology, the addition of fat to the milk, the loss of moisture during the ripening period, and the addition of calcium chloride to pasteurized milk (FOX et al., 2017).

The calcium content in traditional Camembert cheese is 388 mg/100 g (USDA, National Nutrient Database for Standard Reference, 2018). The variability of our results indicated the presence and absence of added calcium chloride in cheese processing (Table 1). The high levels of Ca

(> 388 mg/100 g) may have occurred due to factors such as the animals' feed and possible errors in the preparation of the Ca solution (FOX et al., 2017). The addition of calcium chloride (0.02 g/L) to pasteurized milk helps to achieve constant firmness in the gel and aids the coagulation time (LUCEY et al., 2003).

The salt content in white mold surfaceripened cheeses is 1.5-2.0% (w/w) (LECLERCQ-PERLAT, 2011). The sodium content presented great variability (up to 80.3%, Table 1) but was below the values established for Brie-type cheese (842 mg/100 g) and Camembert-type (629 mg/100 g) cheese (USDA, National Nutrient Database for Standard Reference, 2018). This high variability is related to the lack of control of the salting process. Low concentrations of salt may: (1) affect the control of lactic bacteria growth; (2) not be effective to prevent undesirable microbial growth by killing or limiting the growth of foodborne pathogens and spoilage microorganisms; (3) affect the taste; and (4) modify the water binding capacity with casein, which affects the stability and textural properties of the cheese (EL-BAKRY, 2012).

The whiteness index (WI) can be used as an indicator of the complete recoating of the mycelium on the surface of cheese (JUDACEWSKI et al., 2016). The mean WI values for the whole cheese and cheese wedges were 69.9 and 90.9, respectively. The lower whiteness index for the cheese wedge was related to the handling and type of packaging (PVC film stretchable).

The texture profiles between the two groups of cheeses presented high amplitude (Table 1). At the beginning of ripening, fresh cheeses present a firm and brittle texture (hardness of >10 N) and after ripening they become soft (LECLERCQ-PERLAT, 2011). The mean values for the whole cheese and cheese wedges indicated that they were partially ripened (Table 1). However, the minimum values for hardness (< 3.8 N) indicated that the cheeses were soft. The ripening time may have influenced the high variability in the hardness values (up to 91%, Table 1) that were reported. The softness of these cheeses was due to the increase in pH levels (4.6 to 6.8), which was caused by the surface microbiota flora that solubilize the calcium phosphate between the units of caseins, enabling the hydrolysis of the proteins (β -casein, k-casein, α_{s1} -casein and α_{s2} -casein) by the rennet (LUCEY et al., 2003; SPINNLER, 2017). The diffusion of fungal proteases is limited and can only affect a few superficial millimeters in white mold surface-ripened cheeses. Therefore, proteolysis is not responsible for the softening of this class of cheese (SPINNLER, 2017). The values for cohesiveness, chewiness and resilience were similar for both types of cheese (Table 1).

An important difference between the minimum and maximum values was observed in all evaluated parameters. However, among mean results no significant difference was observed in relation to the majority of the chemical parameters and the texture profile. Therefore, these mean values could be considered the recommended for these cheeses in Brazil.

In our study, *L. monocytogenes* was reported in one sample of whole cheese (5%). The presence of *L. monocytogenes* may be related to the inefficient pasteurization of milk, or defects in the hygiene of the processing environment and equipment used in the manufacture of cheese (GUATEMIM et al., 2016). According to RYSER & MARTH (1987) and CIBELLI et al. (2008), *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella infantis* can grow in parallel with increased pH levels in white mold surface-ripened cheeses.

In terms of *Salmonella* sp., all the evaluated samples of whole and fractionated cheese presented negative results. The search for coagulase-positive *Staphylococcus* and thermotolerant coliforms in the samples presented satisfactory results (< 10 CFU/g and < 3-120 MPN/g, respectively) in accordance with the norms established by RDC n°.12/2001 (BRASIL, 2001). These results suggested that the hygiene milking, cooling and pasteurization of the milk were well controlled (TRMČIĆ et al., 2016; GUATEMIM et al., 2016).

In addition, multivariate tools were used to evaluate all the variables together and to determine their main effects on the cheeses. Principal component analysis (PCA) was applied to evaluate all the physical, chemical, textural and instrumental color data for the whole cheeses and cheese wedges. The first principal component (PC1) was able to explain up to 21.84% of total variance and PC2 explained 17.05% of total variance, totaling 38.89% (Figure 1). Using a 2D scatter plot it was possible to verify the distinction between the two types of cheese (whole and wedges). The underside of the plot (negative factor 2) mainly contained the cheese wedge samples (Figure 1A), with the differences between the cheeses reflected in higher levels of lactose, pH, protein, diameter and price/kg. The whole cheeses (top side of plot, positive factor 2) were characterized by higher values for hardness, chewiness, acidity, ash, sodium, calcium, height, mean weight and WI (Figure 1B). Multivariate analysis was an efficient tool to differentiate these two groups of cheeses, indicating that they were different.

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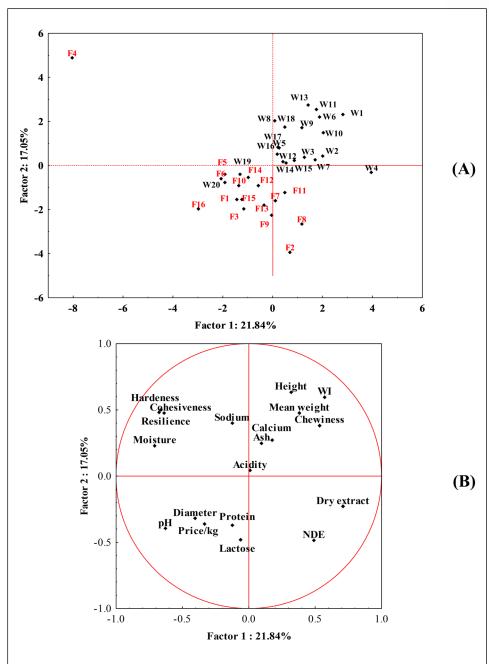


Figure 1 - Principal component analysis (PCA):(A) PCA scores plot; (B) scatter loading plots. Note: Wwhole cheeses; F- cheese wedges; NDE- non-fat dry extract; WI- whiteness index.

CONCLUSION

The whole (Camembert-type) and wedges (Brie-type) cheeses ripened with white mold on the surface were characterized. The results presented high variability (min-max) in all parameters evaluated. This indicated the need to establish identity and

quality standards for the class of cheeses ripened with white mold in Brazil. Among the mean results, no significant difference was observed in relation to the majority of the analyses parameters. These mean values could be considered as recommended for these cheeses. *L. monocytogenes* was reported in a whole cheese sample. The other microbiological parameters

indicated that these cheeses were in accordance with the current legislation. Multivariate analysis was an efficient tool to differentiate between these two groups of cheeses produced in Brazil.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

A.C.P. Pereira, P. Judacewski and G. Coelho performed the analysis in the laboratory. The authors contributed equally in the writing of the manuscript.

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