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ORIGINAL ARTICLE

Influence of basil (*Ocimum basilicum* Lamiaceae) addition on functional, technological and sensorial characteristics of fresh cheeses made with organic buffalo milk

Jessyca C. R. Ribas[1] · Paula T. Matumoto-Pintro[1,2,3] · Ana Carolina P. Vital[1] · Bianka R. Saraiva[3] · Fernando A. Anjo[3] · Ruth L. B. Alves[2] · Nadine W. Santos[1] · Erica Machado[1] · Bruna C. Agustinho[1] · Lúcia M. Zeoula[1]

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Abstract The inclusion of basil in the development of fresh cheeses made with organic buffalo milk was studied. The treatments were: 0 (without basil), 2.5, 5.0 and 7.5 g dried basil/kg of cheese. The cheeses were stored under refrigeration at 4 ± 1 °C during 21 days. The addition of basil did not modify fat, protein, moisture and mineral content of cheeses. The total polyphenol content and antioxidant activity of cheeses increased with basil addition at day one, with a significant reduction in cheeses with 21 days of storage. Cheeses with basil presented higher antioxidant activity and lower pH. The inclusion of basil changed hardness and chewiness, but not influenced springiness and cohesiveness. The microstructure was less homogeneous in cheeses with basil. Cheese with 2.5 and 5.0 g dried basil/kg cheese were preferred by consumers. Thus, the basil improves functional and modify technological characteristics of fresh cheeses and presented good acceptability.

Keywords Natural antioxidant · Polyphenols · Sensory analysis · Microstructure

✉ Paula T. Matumoto-Pintro
ptmpintro@gmail.com

[1] Programa de Pós-graduação em Zootecnia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, Paraná 87020-900, Brazil

[2] Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, Paraná 87020-900, Brazil

[3] Programa de Pós-graduação em Ciência de Alimentos, Universidade Estadual de Maringá, Av. Colombo, 5790, Maringá, Paraná 87020-900, Brazil

Introduction

A functional food, in addition to their nutritional value, provides beneficial effects to body related to health and well-being or reducing diseases risk (Roberfroid 2002). A food can be functional by increasing concentration, adding or improving bioavailability of bioactive ingredient, such as: probiotics, fibers, phytochemicals, vitamins, minerals, herbs, omega-3s, peptides-proteins (Arvanitoyannis and Van Houwelingen-Koukaliaroglou 2005; Roberfroid 2002).

Basil (*Ocimum basilicum* Lamiaceae) is rich in polyphenols, antioxidant, antimicrobial and antifungal properties (Carocho et al. 2016). The basil branches mainly contain fibers, essential oils and minerals, such as nitrogen, calcium, potassium and magnesium. Basil leaves have significant amounts of carotene, vitamin B (1, 2, 3), vitamin C, minerals (calcium, phosphorus and iron), polyphenols and essential oils (Dumbravă et al. 2012). Thus, can be added to foods as a functional ingredient, such as dairy products.

Cheese is a product derived from milk, acquired by milk coagulation, where the final product pass by different operations starting by adding rennet to milk, then cutting the coagulum, draining the whey, shaping, which may include further steps, in which fat and casein are concentrated between six and twelve times, depending on cheese variety (Robertson 2012). Fresh cheeses are very appreciated by consumers due to its physical characteristics: soft, different textures—with or without mechanical eyes, whitish color, mild acid taste and mild odor (Brasil 2004).

Phenolic compounds, secondary products of plant metabolism, have been suggested as bioactive compounds, due to their antioxidant capacity and beneficial effects on human health (Han et al. 2011a). Additions of seasoning plants, rich in phenolic compounds, in cheeses have already been reported (Asensio et al. 2015).

These phenolic compounds have strong interaction with milk caseins by hydrophobic interactions, hydrogen bonds, ionic and covalent bonds (Han et al. 2011a). In this way, the addition of plants extract in cheese may affect their physical–chemical, structural and texture characteristics, and also sensory characteristics (Giroux et al. 2013; Han et al. 2011b). However, there are few studies that show the effects of plants polyphenols in fresh cheeses. Thus, the objective of this study was characterize the bioactive compounds of basil, the effects of basil addition on chemical composition, texture, microstructure, total phenolic content, antioxidant activity and sensorial acceptance of fresh cheeses.

Materials and methods

Reagents

Calcium chloride, Folin-Ciocalteu, sodium carbonate, gallic acid, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS), potassium persulfate, 2,2-diphenyl-picrylhydrazyl (DPPH) and phosphate buffered saline (PBS) standards of caffeic acid, ferulic acid, gallic acid, rosmarinic acid and quercetin were obtained from Sigma Aldrich (Spruce Street, St. Louis, USA). Protease was from HA-LA (Hansen A/S, Denmark). Other reagents used were of analytical grade.

Milk

The milk from Murrah buffaloes raised in organic system and mechanized production was obtained from Instituto Agronômico do Paraná (IAPAR, Lapa, PR). The milk was pasteurized at 65 °C for 30 min and refrigerated at 4 ± 1 °C for 1 day.

Basil

Basil (*Ocimum basilicum* L.) was purchased from local farmer in Maringá/PR (Latitude: 23°25′38″South, Longitude: 51°56′15″West). Branches and leaves were sanitized with water and sodium hypochlorite (200 ppm, 5 min) and dried in an oven at 55 °C for 24 h. The basil branches and leaves (26.85 and 73.15%, respectively) of were ground in a food processor (Cuisinart—Coffee Grinder; DCG-20BK, 130 W of power) and sieved through 60 meshes. Then, was storage (1 week) under refrigerated and dark conditions.

Characterization of milk and basil

To determine the chemical composition of milk, and somatic cell counting the buffalo milk was homogenized and analyzed by the Ekomilk Total equipment (Cap-Lab, São Paulo, Brazil).

The chemical composition of basil was determined according to Association of Official Analytical Chemists (AOAC 1990) for dry matter (930.15), ashes (942.05) crude protein by Kjeldahl method (954.01) and ethereal extract (920.39). Soluble and insoluble dietary fiber (991.43) was determined according to AOAC (1995) and calculated on the basis of dry matter.

Manufacture of fresh cheeses

The manufacturing process of the control cheese was performed using 250 mL of milk heated at 37 °C with addition (0.1 mL) of calcium chloride solution (50%) and protease enzyme (HA-LA) (2.5 mg). The solution was stirred, placed in an oven at 35 °C for 45 min. The cheese mass was cut after 15 min and then molded. The cheeses were kept at rest for 1 h (4 ± 1 °C), and then the turning of the cheese was made. After 30 min the cheeses were packaged in vacuum packer (Sulpack SVC 620 applied during 15 s), to not alter its structure and remained refrigerated at 4 ± 1 °C during 21 days. For cheese with basil, the milk was pasteurized, then the basil powder was added, mixed with the milk during 10 min, and the same procedure as described for control was repeated. The height and diameter (cm) were measured in each cheese, and the yield was calculated by the percentage of cheese production divided by initial milk volume.

Determinations of bioactive compounds

Determinations of basil phenolic compounds by HPLC

The phenolic compounds of basil were extracted with methanol (60%) in a ratio of 1:10 (w/v), after the solution was mixed in vortex (1 min), followed by 10-min in rest (4x). The solution was then centrifuged (3000 rpm, 10 min, 22 °C) and the extract (supernatant) applied to solid phase extraction cartridge (Oasis HLB, Waters Corporation, Milford, USA), eluting with 80% methanol, resulting in final concentration 7.77 mg/mL. Phenolic compounds were identified and quantified using high performance liquid chromatograph (Alliance Waters e2695, Mildford, USA), equipped with separation module, quaternary pump and photodiode detector. The separation was obtained with reverse phase—C18 column (ACE3 C18-AE, 150 mm × 4.6 mm × 5 µm) at 40 °C. The mobile phase was water in 0.1% formic acid (solvent A) and acetonitrile in 0.1% formic acid (solvent B) in gradient according to Lu et al. (2014) with flow rate of 0.8 mL/min. Detection of compounds was performed between 280 and 330 nm and identified by comparison to UV spectrum of commercial standards. Quantification was performed using standards of caffeic acid, ferulic acid, gallic acid, rosmarinic acid and quercetin. Unknown compounds that presented UV spectrum similar to the UV spectrum of quercetin were quantified as quercetin equivalent. Unknown peaks with UV spectrum similar to gallic acid were quantified as gallic acid equivalent.

Determinations of phenolic compounds

Extract preparation Basil (1:20 w/v), all cheese treatments (with and without basil) (1:10 w/v), and serum (1:10 w/v) were mixed with methanol (100%) for 10 min and centrifuged (3000 rpm) for 15 min. Supernatant was used for analyzes.

Total phenolic content (TPC) The TPC was measured in basil, and cheeses with 1 and 21 days of storage and in cheese serum by Folin–Ciocalteau method (Singleton and Rossi 1965). An aliquot (125 µL) of extract were mixed with (125 µL) Folin-Ciocalteu solution (1:1 deionized water) and (2.250 mL) sodium carbonate (28 g/L). Samples remained in the dark for 30 min and the absorbance was read at 725 nm in a spectrophotometer (Evolution™ 300 e Thermo Scientific). Gallic acid was used for the standard curve and results were expressed in mg of gallic acid equivalent (GAE)/g of sample.

Antioxidant activity (AA)

The AA was determined on basil and also on cheeses with 1 and 21 days of storage with different methods: ABTS free radical scavenging described by Re et al. (1999), DPPH free radical scavenging (Li et al. 2009), which the results were expressed as percentage and iron reduction power (FRAP) (Zhu et al. 2002) expressed in mg gallic acid equivalent (GAE)/g of sample.

Cheese chemical composition

Moisture, crude protein, fat and mineral matter were analyzed in cheeses with 3 days of storage; pH and titratable acidity with 1, 7, 14 and 21 days of storage.

Moisture was determined by freeze-drying (Seligmann and Farber 1971) during 24 h. Ashes (942.05) and crude protein by Kjeldahl method (954.01) was determined according to AOAC (OMoA 1990). Fat content was performed by extraction with chloroform, methanol and water (2:2:2) according to Bligh and Dyer (1959).

The pH was determined using a previously calibrated digital pHmeter (Testo 205), which was measured at three distinct points in each sample. Titratable acidity was measured as per the method given by Instituto Adolfo Lutz (2008).

Cheese instrumental analyses

Color was evaluated in cheese and basil by CIELAB color scale, measuring the L* (100—white; 0—black), a* (+, red; −, green) and b* (+, yellow; −, blue) parameters using a Minolta Chroma Meter CR-400 colorimeter with illuminate D65 as a reference in three different points of the same cheese with 1, 7, 14 and 21 days of storage.

The texture profile analyses (TPA) was performed in cheeses with 1, 7, 14 and 21 days of storage, samples were taken from refrigerator at the time of measurement, weighed and desorbed. The Brookfield texture analyzer-CT III equipment (Engineering Laboratories, INC., Middleboro, MA, USA) was used with the following configurations: TPA; Test speed: 1 mm/s; Compression distance 5 mm; Probe TA4 cylindrical. The whole cheese was used. The variables measured were: hardness, chewiness, cohesiveness and springiness.

Cheese structure

For scanning electron microscopy (SEM), cheeses with 10 days of storage were used, due to their stability. The preparation of cheeses was carried out according to methodology described by Lobato-Calleros et al. (2002) with modifications. Samples with 0.5 cm diameter and height were fixed in glutaraldehyde buffer solution (2%) for 6 h, and then dehydrated in ethyl alcohol with increasing concentrations (50, 60, 70, 80, 90 and 100%) where they remained 30 min in each solution and then placed in acetone for 1 h. Subsequently, the samples were frozen in liquid nitrogen and freeze-dried during 24 h. Samples were mounted on aluminum stubs and coated with a gold layer (Spotter coater, Baltec, SCD 050). Observations were made using a scanning electron microscope (SEM) (Quanta 250—FEI Company) at 15 kV and a magnitude of 5000x.

Cheese sensory analyzes

Sensory analysis was performed on cheeses with 2.5, 5.0 and 7.5 g dried basil/kg of cheese after 24 h of storage at 4 ± 1° C. The salting of cheeses was made before the sensorial analysis through brine with 15% of salt (NaCl), using immersion during 10 min. The sensorial analysis was approved by Research Ethics Committee of State University of Maringá (CAAE: 62124616.6.0000.014). The samples were served coded with random three-digit numbers.

Acceptability of cheeses was evaluated according to methodology described by Meilgaard et al. (1988) by 101 untrained consumers, who consented voluntarily to realize this analyze. Attributes evaluated were: flavor, color, texture, aroma and overall appearance using a nine-point hedonic scale, semi-structured, ranging from 1 = extremely disagreeable and 9 points = extremely liked.

Statistical analysis

The results were assessed by analysis of variance using the general linear model (GLM) with SPSS (v.15.0) (IBM SPSS Statistics, SPSS Inc., Chicago, USA) for Windows. Means and standard deviation were calculated for each variable. Basil concentration in cheese (0, 2.5, 5.0 and 7.5 g dried basil/kg cheese) and storage time were considered fixed factors in a factorial design, with three replicates per treatment for each analysis. The experiment was repeated three times. When differences were statistically significant, a Tukey test was performed with statistical significance set at $P = 0.05$.

Results and discussion

Basil characterization

The content of dry matter, ethereal extract, insoluble dietary fiber and soluble dietary fiber 11.86, 3.24, 38.86, and 12.05 g/100 g DM, respectively. Mineral content (14.25 g/100 g DM) was close to *Ocimum gratissimum* (13.67 g/100 g DM) observed by Khalid (2006). The crude protein concentration (8.56) was below to 20.18 g/100 g DM and 20.15 g/100 g DM reported on leaves, from Akure—Nigeria, of *O. gratissimum* and *O. basilicum* respectively (Ifesan et al. 2006). These differences in protein content are probably due to inclusion of basil branches beyond to leaves, with an increase in others components such as fiber content, reducing the protein contents, characterized by dilution effect. Differences in composition may also be related to the plant maturation time and place of cultivation.

Chromatogram of basil extract is shown in Fig. 1. In relation to the compounds detected, four were identified by commercial standards: caffeic acid, ferulic acid, rosmarinic acid and quercetin (Fig. 1). Concentration of caffeic acid (2.38 mg/100 g DM) was less than 14 mg/100 g DM of Purple Ruffles (McCance et al. 2016). Values of ferulic acid (71.82 mg/100 g DM) were lower than 510 mg/100 g DM of *O. basilicum* L. found by Ghasemzadeh et al. (2016). Also, there was lower concentration of rosmarinic acid (117.82 mg/100 g DM) than 683 mg/100 g DM for Iranian green basil and 499 mg/100 g DM for Genovese basil (Luna et al. 2015). The amount of phenolic may vary according to plant nutrition, maturity, plant area analyzed, temperature, and differences between cultivars that should be considered, it is probable that whole plant used (leaves and branches) influenced in these values.

The TPC value was 100.65 mg GAE/g DM measured by Folin–Ciocalteau method. The TPC found, are higher than 17.58 mg GAE/g DM found in basil leaves in *O. basilicum* and *Ocimum americanum* hybrid (Kwee and Niemeyer 2011). Possibly, presence of lignins and tannins in basil branches may have increased the content of total polyphenols in this study.

The antioxidant capacity of basil in reducing Fe(III) through electron donation was 69.9 mg GAE/g, and the antioxidant capacity measured by free radical scavenging (DPPH and ABTS) revealed greater sequestration of free radical ABTS (76.3%) in relation to sequestration of radical DPPH (44.9%).

Cheese characterization

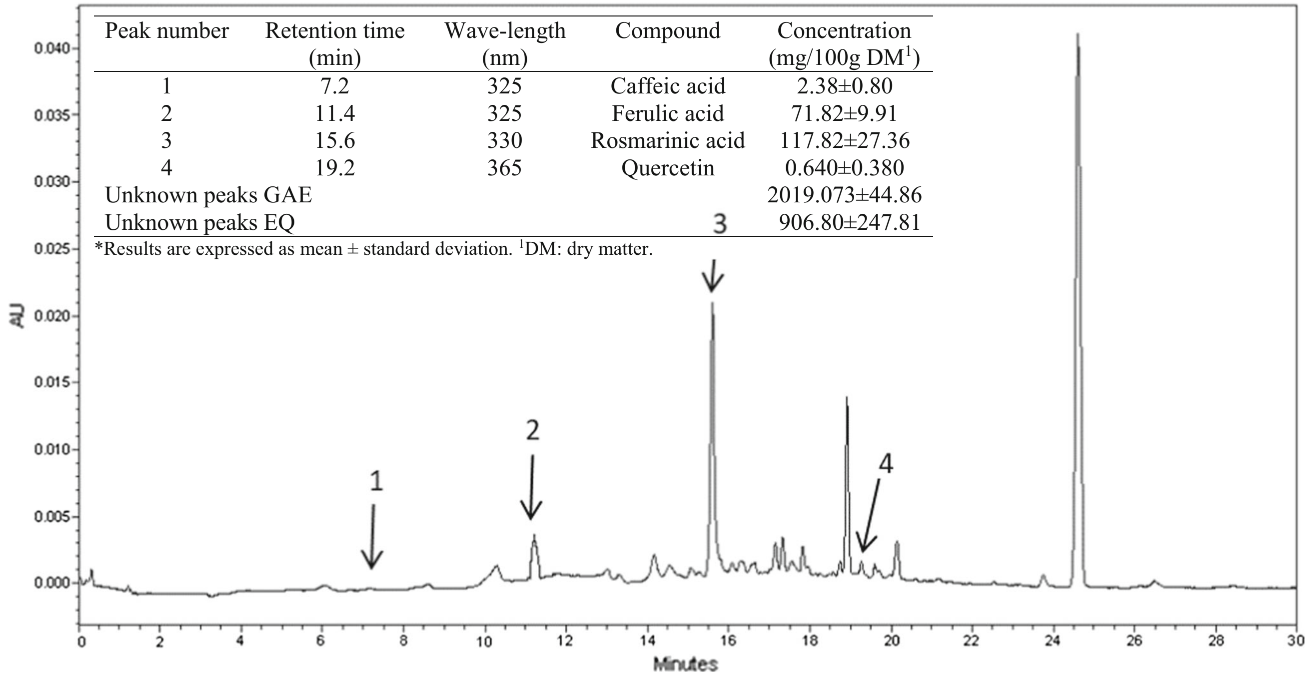
Chemical composition and yield of cheese

Milk presented values of 7.12 for fat, 3.98 for protein, and 4.15 g/100 g for lactose, a pH of 6.94 and 90 cel/mL of somatic cell count. The inclusion of basil did not alter ($P > 0.05$) the amount of mineral matter (44.2–44.3 g/kg), protein (236.7–253.4 g/kg) and fat (422.1–485.0 g/kg) of cheeses. Cheddar cheeses enriched with green tea extract did not present differences in total protein, fat, salt and calcium content between formulations (Giroux et al. 2013). In cheeses made with basil, there was slight decrease in moisture, due to higher syneresis at the manufacture time, however, it was not significate ($P > 0.05$). The presence of polyphenols may slightly reduce pH of clot by stimulating contraction of cheese protein matrix, reducing amount water in protein network (Han et al. 2011b).

A higher syneresis was observed in cheese with basil, thus control showed the higher yield (39.81 ± 0.14%), followed by the others treatment, 2.5, 5 and 7.5 g dried basil/kg cheese (36.99 ± 0.83, 35.18 ± 0.67 and 35.10 ± 1.01%, respectively). Cheeses had initial height of 2.06 ± 0.06 cm and 6.99 ± 0.06 cm in diameter which reduced, respectively, 20% and 5% with 21 days of storage (1.64 ± 0.08 cm in height and 6.64 ± 0.08 cm in diameter). These reductions were due to water losses during storage time. The average weight of cheeses with 1 day of storage (91.53 ± 5.14 g) reduced 30% with storage (65.26 ± 2.71 g).

pH and titratable acidity

The pH influences many cheese properties, altering the solubility of minerals, moisture content, extent and pattern of proteolysis and their interactions, consequently affecting texture and microstructure of cheeses (Pastorino et al. 2003), and polyphenols may reduce pH of milk depending



Peak number	Retention time (min)	Wave-length (nm)	Compound	Concentration (mg/100g DM[1])
1	7.2	325	Caffeic acid	2.38±0.80
2	11.4	325	Ferulic acid	71.82±9.91
3	15.6	330	Rosmarinic acid	117.82±27.36
4	19.2	365	Quercetin	0.640±0.380
Unknown peaks GAE				2019.073±44.86
Unknown peaks EQ				906.80±247.81

*Results are expressed as mean ± standard deviation. [1]DM: dry matter.

Fig. 1 Chromatogram of basil extract at 275 nm

on the compound and the concentration added (Han et al. 2011b).

In this study, the pH of cheese reduced and acidity increased with an increase in basil concentration (Table 1), probably due to the presence of polyphenols and organic acids like oxalic, quinic, malic, shikimic and citric acids (Carocho et al. 2016). The phenolic compounds present in basil may have degraded to monomeric phenolic acids with different concentrations of acid (pKa) through cleavage or oxidative hydrolysis (Wegrzyn et al. 2008), reducing pH of cheese.

Color

In the development of new products, the visual presentation is essential for consumer acceptability. The basil presented 43.86 of luminosity, − 2.025 of redness (a* value) and 17.30 of yellowness (b* value). The color of cheeses made with buffalo milk and basil addition was determined during 21 days of storage and presented in Table 1.

Addition of basil resulted in greater changes of color. Cheese with basil presented lower luminosity, colors tending to green for the lowest basil concentration addition, and green color for cheeses with 5.0 and 7.5 g dried basil/kg cheese. Also, a more intense yellow color was observed for cheese with basil. With storage time, the control cheese presented more color changes, becoming redder and more yellow.

The data showed was in accordance with those found by Giroux et al. (2013), who observed lower luminosity and more yellow intensity for cheddar cheese with addition of green tea extract.

Texture of cheeses

Texture is an important attribute for food sensory acceptance and the parameters measured in cheeses are described in Table 2. Compounds present in basil such as polyphenols and fibers interfered in hardness and chewiness of cheeses. In relation to polyphenols, Giroux et al. (2013) also observed an increased in hardness in Cheddar cheeses enriched with green tea extract. The influence of the polyphenols on the texture occurs indirectly, the reduction of pH (values close to 5.0) in cheeses formulated with polyphenols can reduce moisture of cheeses and alter the concentration of soluble calcium (Han et al. 2011b), making cheese less cohesive and less springiness (Pastorino et al. 2003). The lowest pH found in this study was 5.65 which were insufficient to cause great changes in springiness and cohesiveness of the cheeses. It is observed that there was a small reduction of the springiness with the increase of the storage period, indicating small structural breakage of the cheeses (Tunick et al. 1993).

Probably in cheeses formulated with basil, part of moisture was absorbed in basil fiber, and thus, increasing hardness. Storage time also influenced hardness and chewiness of cheeses, since with longer storage time there was greater syneresis. The reduction of moisture increases hardness of cheese as the volume of protein fraction increases (Lucey et al. 2003).

Table 1 pH, titratable acidity and color of fresh cheeses made with different concentrations of basil during storage

Treatments					
Day	Control[1]	2.5[2]	5.0[3]	7.5[4]	*P* value
pH					
1	6.42 ± 0.02^{Aa}	6.21 ± 0.01^{Ab}	6.11 ± 0.01^{Ac}	6.07 ± 0.01^{Ac}	< 0.001
7	6.28 ± 0.04^{Ba}	6.16 ± 0.01^{Ab}	6.08 ± 0.01^{Ac}	6.03 ± 0.00^{Ac}	< 0.001
15	6.20 ± 0.02^{Ca}	5.80 ± 0.03^{Bb}	5.71 ± 0.01^{Cc}	5.65 ± 0.01^{Bc}	< 0.001
21	6.05 ± 0.01^{Da}	5.83 ± 0.01^{Bb}	5.75 ± 0.01^{Bc}	5.68 ± 0.02^{Bd}	< 0.001
P value	< 0.001	< 0.001	< 0.001	< 0.001	
Titratable acidity (× 1000)					
1	5.5 ± 1.3^{Cd}	6.4 ± 1.3^{Bc}	7.3 ± 1.2^{Cb}	8.7 ± 1.2^{Da}	< 0.001
7	5.9 ± 1.1^{BCc}	6.7 ± 1.5^{Bc}	7.9 ± 1.2^{Cb}	9.6 ± 1.2^{Ca}	< 0.001
15	6.9 ± 1.2^{Bd}	10.5 ± 1.2^{Ac}	11.9 ± 1.2^{Bb}	13.3 ± 1.4^{Ba}	< 0.001
21	8.9 ± 1.5^{Ac}	11.2 ± 1.2^{Ab}	14.7 ± 1.2^{Aa}	15.6 ± 1.1^{Aa}	< 0.001
P value	< 0.001	< 0.001	< 0.001	< 0.001	
L*					
1	91.21 ± 0.13^{a}	81.73 ± 2.25^{b}	74.13 ± 4.63^{c}	65.67 ± 0.73^{d}	< 0.001
7	91.14 ± 0.21^{a}	81.30 ± 1.82^{b}	74.74 ± 3.28^{c}	67.46 ± 0.77^{d}	< 0.001
15	91.26 ± 0.07^{a}	80.50 ± 2.20^{b}	71.62 ± 0.89^{c}	64.54 ± 3.77^{d}	< 0.001
21	91.65 ± 0.42^{a}	81.55 ± 1.43^{b}	70.22 ± 2.15^{c}	66.11 ± 4.03^{c}	< 0.001
P value	0.426	0.727	0.302	0.659	
a*					
1	1.75 ± 0.08^{Ba}	0.83 ± 0.05^{b}	-0.09 ± 0.21^{c}	-0.48 ± 0.62^{c}	< 0.001
7	1.80 ± 0.14^{Ba}	1.18 ± 0.16^{b}	-0.37 ± 0.11^{c}	-0.43 ± 0.11^{c}	< 0.001
15	1.58 ± 0.06^{Ba}	0.93 ± 0.14^{b}	0.01 ± 0.10^{c}	-0.11 ± 0.35^{c}	< 0.001
21	2.17 ± 0.15^{Aa}	1.14 ± 0.28^{b}	-0.24 ± 0.19^{c}	-0.28 ± 0.29^{c}	< 0.001
P value	0.001	0.215	0.079	0.658	
b*					
1	0.84 ± 0.11^{Bd}	6.14 ± 1.05^{c}	7.71 ± 0.67^{ABb}	10.63 ± 0.50^{ABa}	< 0.001
7	1.18 ± 0.27^{Bd}	5.78 ± 0.14^{c}	7.48 ± 0.38^{Bb}	10.74 ± 0.29^{Aa}	< 0.001
15	1.27 ± 0.09^{Bc}	5.55 ± 1.05^{b}	9.00 ± 0.25^{Aa}	9.18 ± 0.34^{Ba}	< 0.001
21	1.97 ± 0.19^{Ac}	6.34 ± 0.81^{b}	8.98 ± 0.81^{Aa}	10.51 ± 0.92^{ABa}	< 0.001
P value	0.003	0.735	0.019	0.032	

Means with different uppercase letters in the same column have a significant difference ($P < 0.05$). Means with different lowercase letters on the same line have a significant difference ($P < 0.05$). Results are expressed as mean ± standard deviation

[1]Control: cheese without basil

[2]2.5–2.5 g dried basil/kg cheese

[3]5.0–5.0 g dried basil/kg cheese

[4]7.5–7.5 g dried basil/kg cheese

Total phenolic content and antioxidant activity of cheeses

The main objective of adding basil to cheese in this study was to increase the concentration of phenolic compounds. The TPC and antioxidant activity (AA) of cheese are described in Table 3. Control presented 0.39 mg GAE/g of TPC. The quantified phenolic compounds in control may possibly derive from milk used, animal feed, amino acid catabolism or rumen microbial activity (O'Connell and Fox 2001). Cheeses with basil showed an increase in TPC with an increase in basil concentration; however, cheese with 5.0 and 7.5 g dried basil/kg cheese did not showed differences between them, as well as in the retention coefficient (RC), which was calculated by the following formula:

$$RC = \frac{(Initial\ polyphenol\ content\ in\ milk - polyphenol\ content\ in\ serum)}{initial\ polyphenol\ content\ in\ milk}.$$

In relation to AA, control cheese had lower capacity to transfer hydrogen atom and electrons in relation to others

Table 2 Hardness, chewiness, cohesiveness and springiness of cheeses with different concentrations of basil during storage

Treatments	Control[1]	2.5[2]	5.0[3]	7.5[4]	*P* value
Hardness (g)					
1 day	498.33 ± 76.86^{Bc}	600.00 ± 14.14^{Bbc}	731.66 ± 49.07^{Bb}	908.33 ± 35.47^{a}	< 0.001
7 days	585.00 ± 50.00^{ABb}	630.00 ± 13.23^{Bb}	696.66 ± 60.28^{Bab}	815.00 ± 65.57^{a}	0.003
15 days	661.66 ± 120.14^{ABb}	822.50 ± 9.68^{Aab}	868.33 ± 49.33^{Aab}	1111.66 ± 95.11^{a}	0.007
21 days	740.00 ± 0.00^{Ab}	813.33 ± 29.29^{Ab}	910.00 ± 47.69^{Aab}	1110.00 ± 181.93^{a}	0.019
P value	0.045	< 0.001	0.003	0.069	
Chewiness (mJ)					
1 day	16.33 ± 2.23^{Bc}	22.30 ± 3.53^{bc}	26.53 ± 1.95^{ABab}	31.60 ± 1.48^{Aa}	< 0.001
7 days	14.06 ± 0.75^{Bb}	20.23 ± 4.34^{ab}	23.30 ± 2.29^{Ba}	23.30 ± 0.62^{Ba}	0.006
15 days	24.20 ± 0.55^{Ac}	26.20 ± 0.28^{bc}	31.47 ± 2.62^{Aab}	34.10 ± 2.30^{Aa}	0.001
21 days	23.85 ± 0.92^{Ab}	29.93 ± 4.41^{ab}	28.36 ± 0.87^{ABab}	33.00 ± 0.95^{Aa}	0.028
P value	< 0.001	0.088	0.007	< 0.001	
Cohesiveness					
1 day	0.79 ± 0.09	0.89 ± 0.07	0.81 ± 0.01	0.82 ± 0.01	0.392
7 days	0.70 ± 0.05	0.83 ± 0.21	0.81 ± 0.01	0.81 ± 0.07	0.191
15 days	0.81 ± 0.05	0.80 ± 0.01	0.79 ± 0.070	0.77 ± 0.07	0.811
21 days	0.83 ± 0.01	0.85 ± 0.14	0.81 ± 0.03	0.82 ± 0.03	0.931
P value	0.200	0.865	0.829	0.601	
Springiness (mm)					
1 day	4.22 ± 0.09	4.25 ± 0.23	4.18 ± 0.05	4.30 ± 0.04	0.608
7 days	3.96 ± 0.53	3.89 ± 0.32	3.88 ± 0.18	4.03 ± 0.32	0.949
15 days	4.05 ± 0.16	4.07 ± 0.05	4.16 ± 0.16	4.10 ± 0.11	0.803
21 days	3.95 ± 0.11	4.05 ± 0.35	3.93 ± 0.08	4.05 ± 0.19	0.867
P value	0.718	0.644	0.084	0.392	

Means with different uppercase letters in the same column have a significant difference ($P < 0.05$). Averages with different lowercase letters on the same line have a significant difference ($P < 0.05$). Results are expressed as mean ± standard deviation

[1]Control: cheese without basil

[2]2.5–2.5 g dried basil/kg cheese

[3]5.0–5.0 g dried basil/kg cheese

[4]7.5–7.5 g dried basil/kg cheese

($P < 0.05$). In relation to DPPH radical scavenging, the AA of cheeses with basil was higher than control in both times analyzed (1 and 21 days) and differences were observed between cheeses with basil at 21 days of storage. Regarding to ABTS radical scavenging, at day one, control showed the lower antioxidant activity, followed by cheese with 2.5 g dried basil/kg cheese, and the other two concentrations (5.0 and 7.5 g dried basil/kg cheese), which did not differ between them. At day 21 of storage, the AA of control and cheese with 2.5 g dried basil/kg cheese was similar, and cheese with 7.5 g dried basil/kg cheese showed the higher AA.

The iron reduction power was higher for cheeses made with 7.5 g dried basil/kg cheese on first day of storage and control had the lowest AA. In relation to day 21, control also presented the lowest AA, and cheese with basil showed similar antioxidant activity.

At 21 days of storage, all cheeses presented a reduction in TPC and AA. This reduction in AA was probably due to the formation of a complex between polyphenols and milk proteins (Lamothe et al. 2014).

Microstructure of cheeses

The scanning electron microscopy (SEM) of the cheeses (Fig. 2) shows internal structure of cheeses with a protein matrix containing fat globules interspersed with dark areas. Control cheeses presented more homogeneous microstructure, with more closed structure, small casein aggregates, small fat globules, smaller and regular dark areas (Fig. 2a). Cheeses formulated with basil had not a uniform structure, with less defined casein networks and larges dark areas (Fig. 2b–d).

Table 3 Total phenolic content and antioxidant activity of fresh buffalo cheeses made with basil

Treatments					
Storage	Control[1]	2.5[2]	5.0[3]	7.5[4]	*P* value
Total phenolic content (mg GAE[5]/g cheese)					
1 day	0.39 ± 0.01^{Ac}	0.58 ± 0.00^{Ab}	0.61 ± 0.02^{Aa}	0.61 ± 0.02^{Aa}	< 0.001
21 days	0.23 ± 0.00^{Bc}	0.32 ± 0.02^{Bb}	0.37 ± 0.02^{Ba}	0.37 ± 0.02^{Ba}	< 0.001
P value	< 0.001	< 0.001	< 0.001	< 0.001	
DPPH radical scavenging (%)					
1 day	13.08 ± 0.47^{Ab}	26.07 ± 1.05^{Aa}	27.97 ± 2.81^{Aa}	28.88 ± 1.15^{Aa}	< 0.001
21 days	4.86 ± 0.31^{Bc}	19.24 ± 0.53^{Bb}	20.42 ± 1.64^{Bab}	21.20 ± 0.44^{Ba}	< 0.001
P value	< 0.001	0.001	0.016	< 0.001	
ABTS radical scavenging (%)					
1 day	12.03 ± 0.11^{Ac}	37.46 ± 4.36^{Ab}	49.36 ± 0.60^{Aa}	53.03 ± 0.68^{Aa}	< 0.001
21 days	8.56 ± 1.96^{Bc}	11.23 ± 1.52^{Bbc}	14.50 ± 2.88^{Bab}	15.80 ± 1.18^{Ba}	0.008
P value	0.038	0.001	< 0.001	< 0.001	
Iron reduction power (mg GAE/g cheese)					
1 day	0.73 ± 0.01^{Ad}	0.87 ± 0.01^{Ac}	1.00 ± 0.00^{Ab}	1.13 ± 0.00^{Aa}	< 0.001
21 days	0.35 ± 0.01^{Bb}	0.41 ± 0.04^{Ba}	0.39 ± 0.01^{Ba}	0.42 ± 0.00^{Ba}	0.013
P value	< 0.001	< 0.001	< 0.001	< 0.001	

Means with different uppercase letters in the same column are significantly different ($P < 0.05$). Means with different lowercase letters on the same line are significantly different ($P < 0.05$). Results are expressed as mean ± standard deviation

[1]Control: cheese without basil

[2]2.5–2.5 g dried basil/kg cheese

[3]5.0–5.0 g dried basil/kg cheese

[4]7.5–7.5 g dried basil/kg cheese

[5]*GAE* gallic acid equivalent

The addition of catechins to full-fat cheese also demonstrated an arrangement of non-homogeneous aggregates, causing rearrangements and heterogeneous pattern of casein micelles (Rashidinejad et al. 2016). The microstructure of rennet-induced milk gels containing polyphenols from grape extract, green tea extract and dehydrated cranberry powder showed to be rough, granular, and less branched structure when compared to the control (Han et al. 2011a).

Sensory analysis

The sensory analysis is very important in the elaboration of new products. Cheeses were evaluated by 101 tasters and results are described in Table 4. The notes ranged from 6 (liked slightly) to 7 (liked moderately). Considering the basil concentration, cheeses with 2.5 and 5.0 g dried basil/kg cheese had the best notes regarding to color, texture, taste, overall acceptability and buy intention. In relation to odor, differences were not observed and notes were above 7 for all cheeses.

Cheeses elaborated with 7.5 g dried basil/kg cheese received the lower notes, probably due to the high concentrations of basil, resulting in a color, flavor and odor more accentuated. Besides that, a sandy texture with greater degree of granularity was observed in this cheese, which decreased the acceptability.

The addition of bioactive compounds in foods can lead to a bitterness flavor and negatively affect color, taste and texture of foods (Giroux et al. 2013), which decreases consumer acceptability, and limit the use of extracts or powders in higher doses.

Conclusion

The basil addition in milk to manufacture cheese increased the total phenolic content and antioxidant activity of the cheeses. Bioactive compounds from basil reduced pH, changes the color, texture and microstructure of cheeses. The greatest technological changes occurred especially in cheeses formulated with 5.0 and 7.5 g dried basil/kg

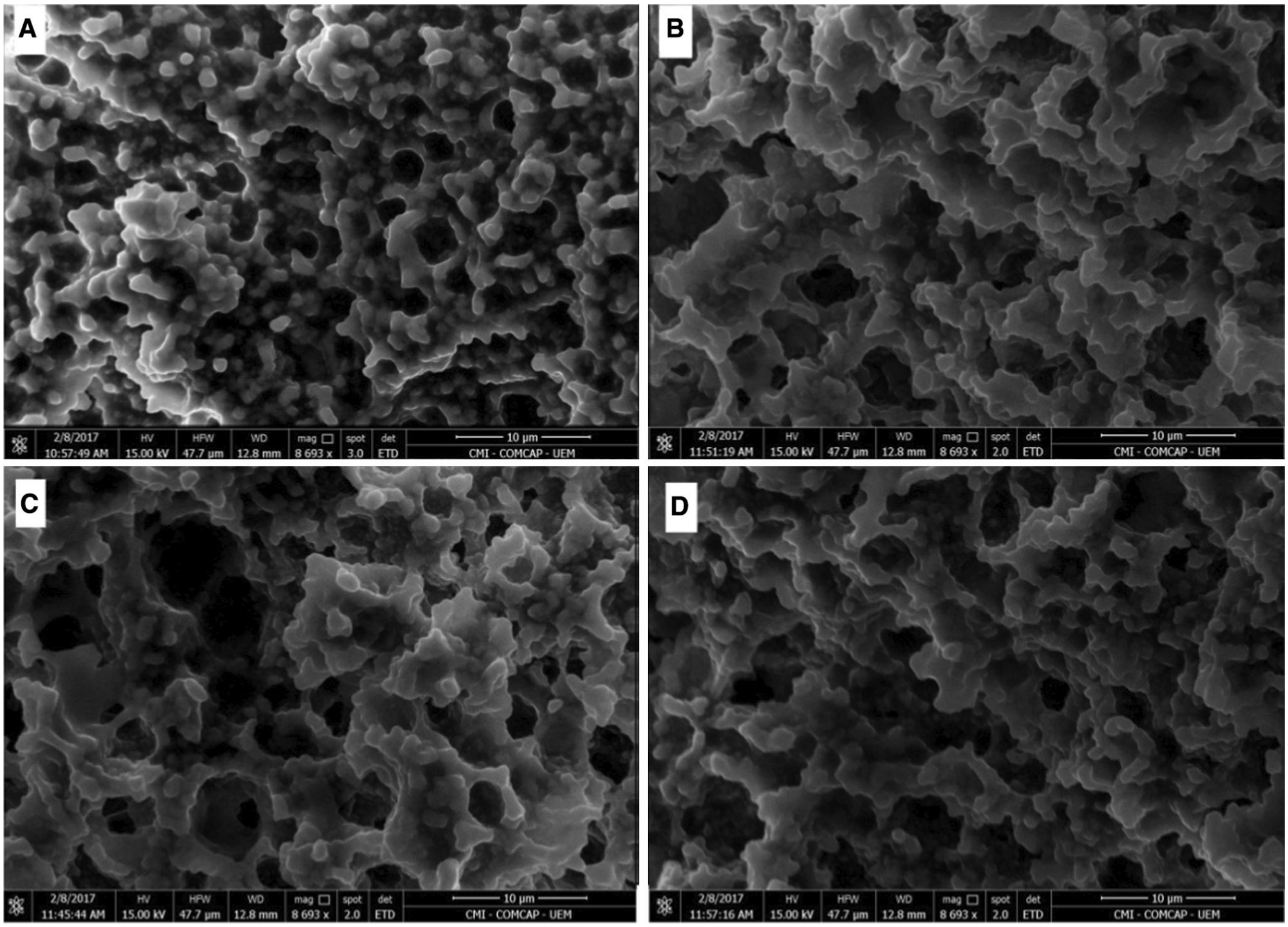


Fig. 2 Scanning electron microscopy images of cheese. **a** Control cheese (without basil); **b** cheese with 2.5 g dried basil/kg cheese; **c** cheeses with dried 5.0 g basil/kg cheese; **d** cheese with 7.5 g dried basil/kg cheese

Table 4 Sensory analysis of fresh cheeses made with buffalo milk and basil

Treatments	2.5[1]	5.0[2]	7.5[3]	*P* value
Color	7.49 ± 0.13[a]	7.24 ± 0.13[a]	6.64 ± 0.16[b]	< 0.01
Odor	7.39 ± 0.13	7.57 ± 0.12	7.32 ± 0.11	0.353
Texture	7.82 ± 0.10[a]	7.52 ± 0.12[ab]	7.18 ± 0.13[b]	0.001
Flavor	7.53 ± 0.12[a]	7.32 ± 0.15[ab]	6.86 ± 0.14[b]	0.003
Overall acceptability	7.63 ± 0.10[a]	7.38 ± 0.12[a]	6.95 ± 0.14[b]	0.001
Buy intention	2.45 ± 0.64[a]	2.27 ± 0.73[a]	1.90 ± 0.79[b]	< 0.001

Means with different lowercase letters on the same line are significantly different ($P < 0.05$). Results are expressed as mean ± standard deviation

[1]2.5–2.5 g dried basil/kg cheese

[2]5.0–5.0 g dried basil/kg cheese

[3]7.5–7.5 g dried basil/kg cheese

cheese. The cheeses with basil were well accepted by consumers (notes above 6.64) and the preference was for cheeses with 2.5 and 5.0 g dried basil/kg cheese. The basil may be an interesting ingredient for cheeses formulations because it improves the antioxidant activity and had a good acceptance by consumers. Besides that, the buffalo milk has large amounts of proteins, fat, vitamins, minerals, which improves the nutritional value of the product, associated with the appeal for an organic product, becoming an interesting product for consumption.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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