

Full Length Research Paper

Effect of the addition of water-soluble soybean extract and probiotic culture on chemical characteristics and folate concentration in yogurts produced with goat's milk

Danielle Cristina Guimarães da Silva

Rua Bahia no. 280/201. São Sebastião. Barbacena, Minas Gerais, Brazil.

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Four yogurts from goat's milk were elaborated and water-soluble soybean extract (WSSE) in the supplementation of 14.8 g/L and *Bifidobacterium lactis* probiotic culture at 2% were added during processing. Chemical aspects and folate concentration were evaluated during the preparation for 29 days at 4°C. Regarding protein and fat analysis, treatments with added WSSE presented higher values; the yogurt with added probiotics presented lower values. Regarding the color, the L* and a* parameters showed no significant differences among the yogurt treatments, the b* parameter values were higher for treatments with added water-soluble soybean extract. The determination of minerals: calcium, iron and potassium were higher for yogurts with added soybean extract. For the sodium content, the values found in yogurts were similar (43.52; 39.00; 40.85 and 47.89 mg/100 g of product). The determination of folates in yogurt presented values of 67 mcg/100 g for the goat's milk yogurt, 135 mcg/100 g for yogurt with added WSSE, 180 mcg/100 g for the yogurt with added *B. lactis* probiotic culture and 195 mcg/100 g for the treatment with added WSSE and probiotic culture. From these results, it was verified that the yogurts presented as viable in terms of their chemical aspects during the storage time of 29 days.

Key words: Yogurt, water-soluble soybean extract, lactic acid bacteria, *Bifidobacterium*.

INTRODUCTION

Yogurt is an extremely popular fermented dairy product produced by the fermentation of the milk lactic acid by the addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. It is a very versatile product that suits all tastes and occasions (Isleten and Karagul-Yuceer, 2006). Yogurts have been reformulated to include living strains of *L.*

acidophilus and species of *Bifidobacterium* (Shah, 2000; Maestri et al., 2014). These probiotic cultures are defined as dietary supplements, which benefit those who consume them by maintaining and/or improving the intestinal microflora balance. Probiotics have been credited with the reduction in lactose intolerance, control of intestinal infection, a reduction in the propensity for some carcinomas,

E-mail: daniellenut@hotmail.com.

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flavor and improved nutritional quality of foods containing them, and the influence on the technological properties of milk products (Denipote et al., 2010). According to the Identity and Quality Standards (PIQ) of fermented milks, in the yogurt viable bifidobacteria count there should be at least 10^6 CFU/mL in the final product, until the expiration date (Brasil, 2000).

In relation to other types of milk, goat's milk presents advantages such as smaller sized fat globules, high digestibility (Frazier, 1995), essential amino acid balance which equals or exceeds the World Health Organization recommendations, high calcium, selenium and phosphate content and being rich in vitamins A and B. It is deficient in folic acid; however, the nutritional deficiency of goat's milk can be improved by the lactic fermentation process (Rao et al., 1984). According to Hugenholtz (2008), many lactic acid bacteria seem to produce some vitamins, from which the fermented product is enriched as a result of bacterial production. Fermented milk products are reported to contain high amounts of folates as a result of the additional production of this vitamin via bacteria. Thus, one can carry out natural fortification of dairy products from the choice of viable starter cultures (Bernaud and Rodrigues, 2013).

Despite the numerous benefits of goat's milk, currently there is a widespread rejection of this product leading to its low consumption (Santos, 2011). For Oliveira (2009), among the viable alternatives to stimulate the consumption of this type of milk is its use in the elaboration of milk drinks, yogurts and cheeses. The preparation of these goat's milk-based products, may present significant changes in their rheological properties, such as low consistency and a tendency towards draining, because the raw material used has a slight reduction in casein content, low proportion or absence of α -s1 casein and high degree of dispersion of the casein micelle, and in addition, the goat's milk curd has semiliquid characteristics (Martin-Diana et al., 2003). Therefore, to obtain satisfactory fermented goat's milk products, stabilizer addition is recommended (Lorenzen et al., 2002).

The use of additives in yogurt, to improve consistency and reduce syneresis, is widely exploited in the industry. There are a number of stabilizers with specific properties available on the market, among these, soybean derivatives such as water-soluble soybean extract is of great importance in the preparation of food products, because they improve the nutritional value of product and affect the formation of the gel structure yogurt (Silva et al., 2012).

The chemical composition of soybeans can vary with the climate, soil type, geographic location, varieties and agronomic practices, among other factors, and the derivatives of soy products, such as soy extract, also undergo changes in their mineral content. Despite this susceptibility to change, soy and its derivatives are important sources of minerals such as potassium (K), iron

Table 1. Identification of yogurts according to the addition of water-soluble soybean extract (WSSE) and probiotic culture.

Yogurt	Added WSSE (20%)*	Added probiotic culture (2%)
A	No	No
B	Yes	No
C	No	Yes
D	Yes	Yes

*WSSE addition adjusted to the milk protein content at 20% concentration, resulting in the supplementation of 14.8 g/L WSSE.

(Fe) and calcium (Ca), highlighting the very high K content (Alejandro et al., 2008).

In this context, the present study was conducted to evaluate the chemical characteristics of color and the level of calcium, iron, sodium, potassium and folates in goat's milk-based yogurts with added water-soluble soybean extract (WSSE) and probiotic culture during refrigerated storage.

MATERIALS AND METHODS

Milk physical and chemical analysis

The milk used in the experiment was from a goat herd through the milking of Saanen females, reared in a semi-intensive system, under adequate hygienic condition. After milking, the milk was immediately cooled to 5°C in an expansion tank, transferred to previously sanitized polypropylene drums and transported to the dairy plant where the processing was carried out. Milk and yogurt sampling and analysis were held during the months of February and April.

The physico-chemical analyzes were conducted using milk samples, in triplicate, to ascertain its quality. These analyzes consisted of pH determination by direct potentiometry in a digital pH meter (Instituto Adolfo Lutz, 1985), titratable acidity measurements (potentiometric titration with NaOH 0.1 molL⁻¹ (Brasil, 2006), density, fat percentage, total soluble solids (Brasil, 2006), defatted solids were determined by subtracting the value of the fat from the value of total solids and crude protein percentage (AOAC, 1995) using the 6.38 factor for the calculation.

Elaboration of yogurt

The study included three elaborations of yogurts during the months of November to February. Four liters of goat's milk were used for the production of the four yogurt treatments in each production. The methodology used in this study for the development of the yogurt was based on that described by Tamime and Robinson (1991). The yogurts were prepared and identified by letters according to their processing particularities (WSSE addition adjusted to the milk protein content at 20% concentration, resulting in the supplementation of 14.8 g/L WSSE and probiotic *Bifidobacterium lactis* at 2%), as expressed in Table 1.

After the milk quality analysis, the WSSE provided by Olivebra[®], was added to Treatments B and D. Subsequently, all treatments were pasteurized at 80°C for 30 min and cooled to 43°C. The different combinations of *Streptococcus thermophilus* bacterial cultures and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Grabolab

Brazil[®] at 0.1% and lyophilized *B. lactis* at 2% (Sacco Brazil[®]) were inoculated individually. The fermentation end point was determined when the yogurt reached a pH of 4.6 which occurred about 5 h after incubation. After this procedure, the yogurts were removed from the oven to reach the pH 4.5 and cooled to 15°C for addition of strawberry pulp (5%) and stored at 4°C.

The analyzes for determination of color and the minerals calcium, iron, sodium and potassium were conducted on the 1st, 8th, 15th, 22th and 29th day of the yogurt treatment post-fabrication, the folate determination analysis was performed on the 15th day, post-fabrication. These deadlines were set because the shelf life of yogurt should be around 30 days, during which the product must maintain its own characteristics, provided that it is properly refrigerated (Vedamuthu, 1991).

Chemical analysis of yogurt

Post-acidification analysis, crude protein, ether extract and the fixed mineral residue (ash) of the yogurt treatments were performed in triplicate. The post-acidification was determined by measuring the pH using Quimis[®] potentiometer model Q-400 (Brazil). The protein fraction was obtained by the Kjeldahl method described by AOAC (1995); the ether extract, by AOAC (1995) and the fixed mineral residue determined by incinerating the sample at a temperature of 550°C (AOAC, 1995).

Determination of color

The color was determined by the CIELAB system (International Commission on Illumination) in Minolta[®] CR 310 equipment (illuminant C or D65 and 10° angle) through the color parameters: L* (lightness), a* and b* (chromaticity coordinates) measured in the equipment itself. Yogurt samples were placed in Petri dishes and the analysis was performed in triplicate.

As for the chromaticity coordinates, +a* is in the red direction, -a* is in the green direction, +b* is in the yellow direction and -b* is in the blue direction. L* measures lightness and varies from 100 (one hundred) for perfectly white surfaces to 0 (zero) for black. The center is achromatic, as the values of a* and b* values increase and the point moves away from the center, the color saturation increases (Minolta, 1994).

Mineral analysis

Minerals (calcium, iron, sodium and potassium) were determined by flame atomic absorption spectrophotometry in a Varian[®] Thectron AA5 spectrophotometer. 0.5 g of yogurt samples were weighed on an analytical scale and subjected to digestion with 6 mL of nitroperchloric solution for about 2 h at 140°C. Sample preparation procedures and mineral quantification were performed according to Malavolta et al. (1997). The results were expressed as a percentage.

Determination of folates

The determination of folates in the yogurt was measured after extraction, deconjugation of polyglutamates using a conjugase (γ -glutamyl hydrolase) and subsequent quantification by high performance liquid chromatography (HPLC) by the method validated by Romero and Camargo (2007).

Statistical analysis

The experiment was conducted in completely randomized design in

Table 2. Milk physical and chemical analysis for the goat's milk.

Physical chemical parameters	Mean (SD)**	IN n.37/2000*
Acidity (g lactic acid/100 ml)	0.17 (0.06)	0.13 a 0.18
Density (15/15°C g/mL)	1.030	1.028 – 1.034
Fat (g/100 g)	3.1 (0.4)	3.0
pH	6.68 (0.14)	-
Protein (g/100g)	3.2 (0.20)	Minimum 2.8
Defatted solids (g/100 g)	8.4 (0.12)	Minimum 8.2
Total solids (g/100 g)	11.5 (0.08)	-

*(Brasil, 2000); ** SD- standard deviation.

a 2 x 2 x 5 factorial with 2 soybean extract concentrations, addition of probiotic culture in 2 treatments and 5 storage times, with three repetitions. The treatments were evaluated by analysis of variance, followed by Scott-Knott test at 5% significance level, using the R software.

RESULTS AND DISCUSSION

Milk physical and chemical analysis

The results of the physicochemical analysis of the goat's milk used in the yogurt treatments are shown in Table 2. The average acidity value, fat, protein, density, total solids and defatted solids of the goat's milk were within the standards of the current legislation. According to the results, it was observed that the milk used in the yogurt manufacture meets the standards required by Instruction n.51 (Brasil, 2000), characterizing it as a raw material of good physicochemical quality, which meets the legal standards required for manufacturing yogurts.

These results were similar to those found by Lora et al. (2006). The results found for pH were similar to the values obtained by research done in the state of São Paulo by Gomes et al. (2004) and Richards et al. (2001) evaluated the pasteurized whole goat's milk, and found average total solid values from 12.08 to 12.23%.

Elaboration of yogurt

The preparation of yogurt occurred after fermentation time in an oven at 42°C for five hours, there was no change in the time between the four treatments. From a practical point of view, the fermentation time registered in this study did not exceed the normal time observed in traditional manufacturing processes, which does not preclude the use of WSSE protein.

Chemical analysis of yogurt during storage

The results of the pH of the yogurt treatments showed

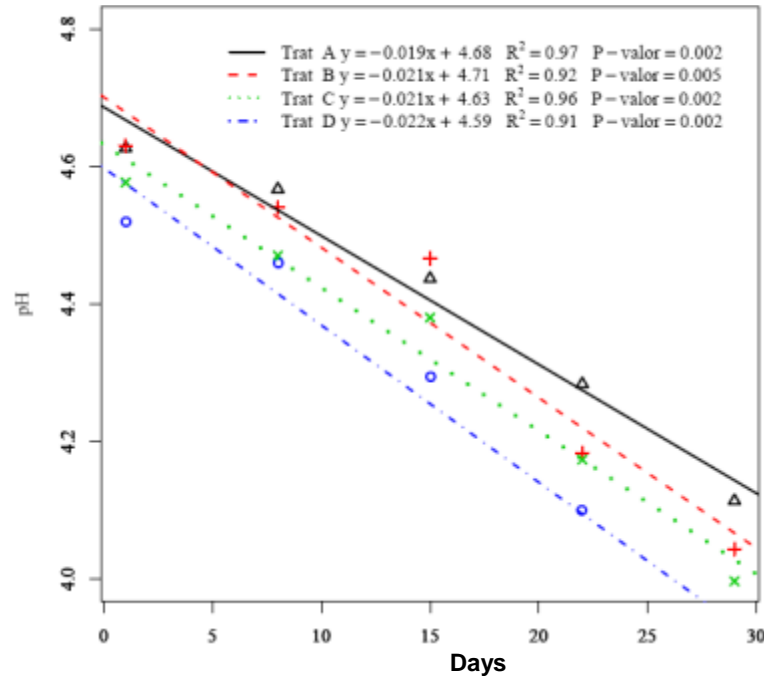


Figure 1. Regression model for the pH as a function of storage time at 4°C in yogurt.

variation (4.6 to 3.8) during storage at 4°C. Treatment A had the highest mean pH (4.45), Treatment B, had a mean pH of 4.38, Treatment C 4.30 and Treatment D showed an average pH of 4.22.

In the evaluation of yogurt, it was noted that there was a significant influence of the treatment time and the pH value according to the graph in Figure 1.

Results similar to the present survey were found by Assumpção (2008) with the production of cow's milk yogurt with added soybean soluble extract. According to Bortolozzo and Quadros (2007), the ideal pH for fermented milks is close to 4.5, since lower values can lead to rejection by consumers and promote clot contraction due to hydration of proteins, causing draining.

In relation to the protein determinations, the yogurt averaged 2.41%, Treatment B averaged 3.2%, Treatment C 2.34% and Treatment D averaged 3.02%. It was observed that the yogurts encoded by the letters A and C had similar protein contents, showing that the addition of the probiotic *B. lactis* caused little proteolysis in these products. In goat's milk yogurt encoded by the letters B and D, the proteins values found were higher than that in the other treatments because of the addition of water-soluble extract during the manufacture.

Lee et al. (1990) evaluated the protein content of soy yogurt supplemented with whey protein concentrate or defatted milk powder. The product supplemented with protein concentrate showed 8.12% protein, while that supplemented with defatted milk powder had 7.28%, both of which had higher protein concentration than the yogurt

developed in our work. The protein content of frozen yogurt for goat's milk supplemented with probiotics evaluated by Alves et al. (2009) showed results similar to those of the present study ($3.0 \pm 0.3\%$), being above the minimum recommended for the product (2.5%).

For the fat content, the average content of Yogurt A was determined at 2.44%, Yogurt B 3.86%, Treatment C 2.35% and Yogurt D 3.63%. The yogurts added WSSE showed higher average fat values of higher fat than the other treatments.

For Thomopoulos et al. (1993), milk fat favorably affects the quality of yogurt, fat stabilizes the protein contraction of the gel, prevents the separation of whey in the final product and affects the sensory perception of the product, which has more soft and creamy texture.

The average values of fixed mineral residue of yogurt with added WSSE were higher than in the other treatments, however, they showed no significant difference among themselves ($p > 0.05$).

Color determination

As can be seen in Table 3, the values of the color determination were not influenced by the addition of the water-soluble soybean extract or the addition of the *B. lactis* probiotic.

Regarding the determination of yogurts color, average values of L* and the chromaticity coordinates a* and b* were not affected by storage time. The mean values of

Table 3. Average color values of samples of goat's milk yogurt with added water-soluble soybean extract and probiotic culture.

Yogurt	L*	a*	b*
A	71.13 ^a	11.16 ^b	0.59 ^c
B	73.22 ^a	11.74 ^b	1.76 ^d
C	72.96 ^a	10.85 ^b	0.68 ^c
D	72.09 ^a	11.38 ^b	1.91 ^d

L* = lightness, +a* = red, -a* = green, +b* = orange, -b* = blue. Means followed by the same letter do not differ statistically among themselves, by Scott-Knott test average, to 5% probability. Yogurts: A (without addition of WSSE and without addition of probiotic culture), B (with addition of WSSE and without addition of probiotic culture), C (without addition of WSSE and with addition of probiotic culture), D (with addition of WSSE and with added probiotic culture).

Table 4. Mean and standard deviation (SD) of calcium, iron, sodium and potassium in samples of goat's milk yogurt with added water-soluble soybean extract and probiotic culture.

Yogurt	Calcium (mg/100 g)	Iron (mg/100 g)	Sodium (mg/100 g)	Potassium (mg/100 g)
	Mean (SD)*	Mean (SD)*	Mean (SD)*	Mean (SD)*
A	74.0 (1.4) ^a	1.0 (0.3) ^a	43.5 (0.6) ^a	160.0 (2.3) ^a
B	116.0 (1.1) ^b	1.9 (0.2) ^b	39.0 (0.2) ^a	166.0 (1.5) ^a
C	62.0 (0.7) ^c	0.9 (0.2) ^a	40.8 (1.1) ^a	132.0 (0.9) ^b
D	86.0 (0.9) ^d	1.3 (0.1) ^a	47.9 (0.8) ^a	190.0 (1.3) ^c

*SD– standard deviation. Means followed by the same letter do not differ statistically among themselves, by Scott-Knott test average, to 5% probability. Yogurts: A (without addition of WSSE and without addition of probiotic culture), B (with addition of WSSE and without addition of probiotic culture), C (without addition of WSSE and with addition of probiotic culture), D (with addition of WSSE and with added probiotic culture).

the brightness parameter and coordinate a* showed no significant difference between the four products analyzed ($p > 0.05$). Only the parameter b* presented higher average values for the treatments with added water-soluble soybean extract, being statistically significant. Research carried out by Moraes (2004), when evaluating traditional brands of strawberry flavor yogurt, found results similar to the present work. The positive values found in the colorimetric analysis of this parameter in yogurts indicate that there was a tendency towards yellow. This direction is caused by the addition of WSSE (Lambrecht et al., 1996). The yellowing of soybeans can vary depending on the cultivar used (hilum color, seed coat color) (Bhardwaj et al., 1999). A similar result was obtained by Ciabotti et al. (2009), on evaluating the color of soymilk and whey based products.

Mineral composition

The concentration of the minerals in the formulations analyzed showed that the addition of soybean extract increased the calcium, potassium and iron in the product, confirming the data described above. Research by Garcia et al. (2006) to determine the mineral content in goat's

milk and dairy products derived from goat's milk produced results similar to those in the present research for the yogurt without soybean extract addition.

Table 4 shows the results of the analysis of minerals found in the yogurts in this study. In this study, the supplementation with water-soluble soybean extract in goat's milk yogurt provided an increase of calcium content (116.0 mg/100 g), however, in the yogurt in which probiotic culture was added, there was a reduction in this value; this fact can be explained by the consumption of nutrients resulting from the metabolism of these microorganisms. It is noteworthy that the probiotics, besides increasing protein and fat digestibility and reducing lactose content, also act by increasing the absorption of some minerals, especially calcium and iron (Gomes and Malcata, 2002).

Goat's milk has higher calcium and potassium levels, which may interfere with the technological characteristics of the product and its derivatives (Haenlein, 2001). However, due to the increase in temperature, pH and inoculation with microorganisms, one can see a reduction in the calcium (Gomes and Penna, 2009) and potassium (Miguel et al., 2010) concentration during the fermentation process. The four yogurt treatments showed statistically

Table 5. Folate content of the samples of goat's milk yogurt with added water-soluble soybean extract and probiotic culture.

Yogurts	Folate (mcg/100 g)
A	67.0 ^a
B	135.0 ^b
C	180.0 ^c
D	195.0 ^d

Means followed by the same letter do not differ statistically among themselves, by Scott-Knott test average, to 5% probability. Yogurts: A (without addition of WSSE and without addition of probiotic culture), B (with addition of WSSE and without addition of probiotic culture), C (without addition of WSSE and with addition of probiotic culture), D (with addition of WSSE and with added probiotic culture).

different average calcium values, showing that the addition of soy extract and bifidobacteria, during fermentation, change the final product.

There was an increase in the iron content in the yogurt supplemented with soybean extract and also the reduction of this mineral in yogurts with added probiotic culture. The iron concentration for samples of yogurts with defatted soybean extract, found by Miguel et al. (2010) were higher than in the present work, probably due to the type of soy extract used for the preparation of the products. This result shows that the iron concentration may vary in accordance with the soybean extract used as a thickener. With respect to sodium, the variation among the yogurt treatments was low, there were no statistical differences between the products. Fuchs et al. (2005), when determining the sodium concentration in yogurt with added soy extract and prebiotics, found values lower than those obtained in the present study. It is noteworthy that goat's milk has lower sodium concentrations than those found in cow's milk (Padua, 2013).

Determination of folates

The determination of folates was conducted in triplicate, on the 15th day after the manufacture of the yogurts. The unfeasibility of folate detection throughout the product shelf life is due to the cost of conducting this procedure. The folate analysis results of the goat's milk yogurt with added water-soluble soybean extract and probiotic culture can be seen in Table 5.

There was a significant difference in the folate concentration values in the analyzed yogurts. All treatments with added WSSE and/or bifidobacteria had higher folate values as compared to the control yogurt. This behavior can be explained by the fact that the WSSE had contributed to the increase in the folate content of the products and also the synthesis of this vitamin by the bifidobacteria.

It can be seen that the yogurt treatment encoded by the letter A had the lowest folate concentration. Research carried out by Rao et al. (1984), in order to evaluate the biosynthesis and use of folic acid in milk fermented by various lactic cultures, showed that the association of *S. thermophilus* and *L. bulgaricus* in fermented milk increased the folate concentration, however, when the author related the folate level with associations of lactic cultures, it was observed that the samples with added *L. bulgaricus* showed lower content of this vitamin. According to Lin and Young (2000), lactic cultures not only synthesize, but also use folates.

It appears also that the C and D treatments with added probiotic culture *B. lactis* had high folate levels. Lin and Young (2000) quantified and evaluated folate stability in milk fermented by lactic cultures of *S. thermophilus*, *L. acidophilus*, *B. longum* and *L. bulgaricus* stored at 4°C, and found that the highest concentration of this vitamin in the fermented milk evaluated was the milk with addition of *B. longum* (98.0 ng/mL).

Biofortification with folate as an approach holds the promise of being cost effective because products with elevated levels of this essential vitamin would provide economic benefits to food manufacturers and increased "natural" folate concentrations would be an important value-added effect, without increasing production costs (Iyer and Toma, 2011).

Conclusions

The levels of the minerals, calcium, iron and potassium were higher for yogurts with added soybean extract. For the sodium content, the values found in yogurts were close to each other. It was also noted that there was an increase in the concentration of folates in yogurts with added probiotic culture.

Conflict of interests

The author(s) did not declare any conflict of interest.

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