PROFILE AND LEVELS OF FREE AMINO ACIDS AND FREE BIOACTIVE AMINES IN FERMENTED MILK

Naiara M. Ciriaco; Guilherme C.L. Reis; Gisela M.M. Machado; Valterney L. Deus, Maria Beatriz A. Gloria*

Laboratório de Bioquímica de Alimentos-LBqA, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil
*Corresponding author: mbeatriz@ufmg.br

ABSTRACT

The profile and levels of free amino acids and bioactive amines in fermented milk were investigated. The five most widely consumed products were obtained immediately after production from the consumer market, and were analyzed throughout shelflife at 15 days intervals for free amino acids and free bioactive amines (UPLC after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate). pH and titratable acidity were also analyzed. Throughout shelf life, fermented milk had mean pH from 4.00 to 4.04 and acidity of 0.98 and 0.99 g lactic acid/100 g for products A and B, respectively. Fermented milk A contained three free amino acids: asparagine, arginine and proline; whereas B had 11: aspartic acid, alanine, arginine, asparagine, histidine, isoleucine, leucine, phenylalanine, tyrosine, threonine and valine. Total amino acids were 4.2 and 17.8 mg/kg, respectively. Both products contained spermine (the only amine detected among nince investigated), at levels of 0.7 mg/kg.

Key words: spermine, polyamines, UPLC, asparagine, arginine.

1. INTRODUCTION

The sales of fermented milk in Brasil are near R$ 400 million per year and two brands are responsible for this market, each one representing 41%. In volume, these companies produce 100 thousand tons of fermented milk per year (Milkpoint, 2018). However five different brands were available in the market of Belo Horizonte, MG, Brazil. These products are mainly directed to children, adult and elderly population. They are sold as probiotics with the intent to help the maintenance of intestinal health.

Fermented milks are prepared by fermentation of milk with the help of starter cultures (Brasil, 2007; Codex 2010). Lactic acid bacteria (LAB) are widely used in the preparation of fermented milk products, and are known to produce specific metabolites during fermentation, such as peptides, fatty acids, and simple sugars, all these contribute toward better digestibility of the fermented foods, and offer nutritional as well as therapeutic qualities (SHIBY & MISHRA, 2013). However, scarce information is available regarding free aminoacids and bioactive amines in fermented milk, which are also components with relevant functional properties. The objective of this study was to investigate the profile and levels of free aminoacids and bioactive amines in fermented milk from the Brazilian market.

2. MATERIAL AND METHODS

Fermented milk samples were purchased from three different locations at the retail market. The samples were analyzed immediately and at 15 days intervals during storage at 9±1°C for up to 60 days. Bioactive amines, amino acid standards and norvaline (internal standard) were purchased from Sigma/Aldrich Co, St Louis, MO, USA). All the reagents were analytical grade and UPLC solvents were chromatographic grade

pH and titratable acidity were determined according to Brasil (2007). Free amino acids and free bioactive amines were analyzed in freeze-dried samples by ultra pressure liquid chromatography and detection at 249 nm after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate – AQC (AccQFluor®, Waters, Milford, MA, USA) (Fiechter et al., 2013). The concentrations of amino acids and amines were calculated by interpolation in the respective analytical curves ($R^2 \geq 0.99$) and the recovery of the internal standard was also used in the calculation. The results were submitted to analysis of variance and the means were compared by the Tukey test at 5% significance, using Minitab version 18.

3. RESULTS AND DISCUSSION

The physico-chemical characteristics of the fermented milk are described in table 1. No significant difference was observed for the physico-chemical characteristics between the two brands investigated. Mean pH values varied from 4.00 to 4.06 and acidity from 0.82 to 1.00 mg lactic acid/100 g for the different products products. There was no significant difference among fermented milk ($p>0.05$) regarding pH and acidity. Every fermented milk is in accordance with the Brazilian legislation (Brasil, 2007) that limits titratable acidity to values from 0.6 to 2.0 mg lactic acid/100 g.

Table 1. pH, and titratable acidity of different brands of fermented milk before expiration date from the consumer market of Belo Horizonte, MG, Brazil

Fermented milk	Values	
	pH	Acidity (mg lactic acid/100 g)
A	4.06±0.08[a] (3.98-4.17)	0.82±0.09[a] (0.73-1.00)
B	4.04±0.02[a] (4.00-4.05)	1.00±0.06[a] (0.90-1.14)
C	4.03±0.05[a] (4.00-4.10)	0.98±0.12[a] (0.80-1.12)
D	4.05±0.07[a] (4.00-4.18)	0.90±0.03[a] (0.85-0.93)
E	4.04±0.04[a] (3.99-4.10)	0.99±0.06[a] (0.94-1.08)

Mean values ± standard deviation with the same superscript in the same line are not significantly different (Tukey test, $p>0.05$).

The types and levels of amino acids in the fermented milk are indicated in Table 2. Among the 20 aminoacids investigated, 11 were detected in the products. Glutamic acid, cystine, glycine, glutamine, methionine, tryptophan and serine were not detected in any sample. The types of amino acidos found in the

fermented milk varied from 3 to 11. Three were detected in products C and D, including argininr, asparaginr and proline. Eight were found in product A; whereas 11 were detected in products B and E.

Table 2. Mean levels of free amino acids in the different fermented milk before expiration date date from the consumer market of Belo Horizonte, MG, Brazil

Amino acids	**Levels (mg/kg)/ fermented milk**				
	A	**B**	**C**	**D**	**E**
Aspartic acid	nd [b]	nd [b]	nd [b]	nd [b]	0.43±0.05 [a] (0.37-0.48)
Alanine	1.14±0.82 [a] (0.39-1.89)	1.68±0.16 [a] (1.56-1.88)	nd [b]	nd [b]	1.88±0.00 [a] (1.88)
Arginine	1.21±0.91 [a] (0.37-2.04)	1.71±1.02 [a] (0.41-2.47)	1.90±0,11 [a] (1.79-2,00)	1.91±0.10 [a] (1.79-2.00)	2.04±0.00 [a] (2.04)
Asparagine	0.87±0.05 [a] (0.82-0.92)	0.87±0.06 [a] (0.81-0.92)	0.73±0,02 [b] (0.71-0,76)	0.73±0.02 [b] (0.71-0.76)	0.87±0.06 [a] (0.82-0.92)
Phenyalanine	nd [b]	1.58±0.46 [a] (1.10-2.03)	nd [b]	nd [b]	1.11±0.00 [b] (1.11)
Histidine	3.76±0.01 [a] (3.76-3.79)	3.78±0.05 [a] (3.76-3.88)	nd [b]	nd [b]	3.76±0.00 [a] 3.76
Isoleucine	1.76±0.21 [a] (1.58-1.98)	1.75±0.16 [a] (1.58-2.08)	nd [b]	nd [b]	0.18±0.02 [b] (0.16-0.20)
Leucine	nd [b]	nd	nd [b]	nd [b]	2.06±0.00 [a] (2.06)
Lysine	nd [b]	0.49±0.34 [a] (0.10-0.98)	nd [b]	nd [b]	nd
Proline	nd [c]	0.79±0.95 [b] (1.97-0.92)	1.56±0.00 [a] (1,56)	1.56±0.00 [a] (1.56)	nd [c]
Tyrosine	0.50±0.14 [a] (0.35-0.63)	0.52±0.10 [a] (0.35-0.62)	nd [b]	nd [b]	0.62±0.00 [a] (0.62)
Threonine	4.34±0.12 [a] (4.08-4.39)	3.72±1.00 [a] (2.37-4.38)	nd [b]	nd [b]	4.38±0.00 [a] (4.38)
Valine	0.48±0.00 [a] (0,48)	0.52±0.03 [a] (0.48-0.56)	nd [b]	nd [b]	0.44±0.04 [a] (0.40-0.49)
Total	14.06±2.26 [b]	17.41±0.33 [a]	4.19±0.13 [c]	4.20±0.12 [c]	17.77±0.17 [a]

Mean values with the same letter in the same line are significantly different (Tukey test, $p<0.05$).

Arginine and asparagine were the only amino acids present in every fermented milk. Aspartic acid and leucine were detected only on product E; lysine was only detected in B; and phenylethylamine only in B an E. Higher total levels of amino acids were found in products B and E (~17 mg/kg), followed by A (14 mg/kg), and by products C and D (~4 mg/kg). This is the first time the profile and levels of free amino acids were compared. Based on these results, the fermented milk had different amino acids profiles and should be selected based on the specific need. For example, products B and E should be avoided by phenylcetonuric individuals (BUENO et al., 2016).

When looking into the contribution of each aminoacid to total levels, in products A and B, threonine was the amino acid which contributed the most (21-31%), followed by histidina (22-27%) and isoleucine (10-12%), and the rest contributed with less than 10%. Products C and D had higher contribution of arginine to total levels (~45%), followed by proline (~37%) and asparagine (17%). Product E, had several amino acids contributing to

total levels, including threonine and histidine (~21 a 25%) and alanine, arginine and leucine contributing with ~11%.

Among the nine amines investigated, only spermine was present. According to Table 3, the levels were similar for both products (0.67 and 0.73 mg/kg, respectively). The presence of this polyamine is relevant as it has important roles in the maturation of the intestinal mucosa (Gloria, 2006). Furthermore is has been reported to have several health promoting properties including antioxidant, antinflamatory, and neuroprotective properties (SASAKI et al., 1996; GABORIAU et al., 2005).

Tabela 3. Mean levels (minimum-maximum) of bioactive amines in different fermented milk before expiration date date from the consumer market of Belo Horizonte, MG, Brazil

Bioactive amines	**Levels (mg/kg)/fermented milk**				
	A	**B**	**C**	**D**	**E**
Spermine	0.68±0.00 [a] (0.68)	0.80±0.16 [a] (0.62-0.93)	0.67±0.01 [a] (0.66-0.68)	0.67±0.02 [a] (0.66-0.68)	0.73±0.06 [a] (0.68-0.79)

Mean values with the same letter in the same line are significantly different (Tukey test, $p<0.05$).

4. CONCLUSION

The different fermented milk had similar pH and titratable acidity and levels of the polyamine spermine. However the profile and levels of amino acids were very different, with three up to 11 amino acids. Furthermore, the levels of the amino acids varied widely among fermented milk. Total levels of amino acids differed significantly, from 4.20 mg/kg up to 17.77 mg/kg. Based on these results productsd B and E are the ones which provide a wider profile and higher levels of amino acids, including the essential ones. The only bioactive amine detected was spermine at similar levels in the different products. This polyamine is a relevant component as in a probiotic as it plays important roles in health and maturation of the intestinal mucosa.

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