XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos
Alimentação: a árvore que sustenta a vida
X CIGR Section IV International Technical Symposium
Food: the tree that sustains life
24 a 27 de outubro de 2016 • FAURGS • GRAMADO/RS

THE EFFECT OF INSTANT ARABICA COFFEE ON THE VIABILITY OF *LACTOBACILLUS ACIDOPHILUS* AND *BIFIDOBACTERIUM BIFIDUM* IN A PROBIOTIC, LACTO-FREE DESSERT

G.Duarte,[1,2] C. O. Fernandes[1], M.M. Lemos,[3] I. Felberg,[4] A. Farah.[1]

1- Programa de Pós-graduação em Nutrição e Núcleo de Pesquisa em Café Prof. Luiz Carlos Trugo - Instituto de Nutrição - Universidade Federal do Rio de Janeiro - - Rio de Janeiro - RJ - Brazil, Telefone: +55(21) 3938 6449 - email: (afarah@nutricao.ufrj.br)
2 - Instituto Federal do Rio de Janeiro - Campus do Rio de Janeiro - Rio de Janeiro - RJ - Brazil, - email: (giselle.oliveira@ifrj.edu.br)
3- Instituto de Microbiologia - Universidade Federal do Rio de Janeiro - Rio de Janeiro - RJ - Brazil, - email: (marco.miguel@micro.ufrj.br)
4 - Embrapa Agroindústria de Alimentos, CEP: 23020-470 - Guaratiba, Rio de Janeiro - e-mail: (ilana.felberg@embrapa.br)

RESUMO – O objetivo deste estudo foi avaliar a viabilidade de bactérias probióticas *Bifidobacterium bifidum* subsp. *lactis* e *Lactobacillus acidophilus* em uma sobremesa sem lactose, à base de extrato de soja, com diferentes concentrações de café (0 a 1,5%), armazenada a 7 ˚C. Quatro formulações produzidas foram avaliadas quanto ao pH, acidez e contagem das bactérias probióticas após 0, 7, 14 e 21 dias de armazenamento. Nem o tempo, nem o teor de café influenciaram significativamente o teor de ácido lático e o pH das amostras estudadas. De acordo com a ANVISA, para que o produto possa ser considerado probiótico, a contagem total de cada cepa por porção (100g) deve ser igual ou superior a 10^8. No presente estudo, este valor foi observado para *L.acidophilus* até 21 dias, e para *B.bifidum* até 7 dias de armazenamento, ambos sem interferência do teor ou percentual de café.

ABSTRACT – The aim of this study was to evaluate the viability of probiotic bacteria *Bifidobacterium bifidum* subsp. *lactis* and *Lactobacillus acidophilus* in a lactose-free soy-coffee dessert, containing different concentrations of instant coffee (0 to 1.5%) during storage, at 7˚C. Four formulations were evaluated for pH, acidity and counting of probiotic bacteria after 0, 7, 14 and 21 days of storage. Neither storage time nor the coffee content significantly influenced the lactic acid content and pH of the samples. According to ANVISA, in order to be considered probiotic, the total count of each strain per serving of the product (100g) must be equal or greater than 10^8. This value was observed for up to 21 days for *L. acidophilus*, and *B. bifidum* within 7 days of storage, both without the interference% coffee.

PALAVRAS-CHAVE: viabilidade; probióticos; café; soja; lactose-free.

KEYWORDS: viability; probiotics; coffee; soybean; lactose-free.

1. INTRODUCTION

As consumers awareness about the link between food and health increases across the world they become more and more interested in foods with health promoting features.

Among the available functional foods, lactose-free and gluten-free products, as well as products containing probiotic microorganisms show promising trends worldwide (Kies, 2014). Soybean is a low cost food, rich in bioactive compounds such as isoflavones, proteins and fibers. Additionally, according to some epidemiological studies, diets including habitual consumption of soy products and/or coffee may reduce the risk of various chronic diseases, most probably due to the antioxidant as well as other regulating effects of phenolic compounds (Farah and Donangelo, 2006; Yan and Spitznagel, 2009).

While coffee consumption in Brazil is high (the country is the largest coffee producer, and second largest consumer in the world) (ABIC, 2014), soy consumption is reduced due to its characteristic beany off-flavour. Fermentation is a technique widely used in soybean products to decrease the beany off-flavour and improve consumer acceptance (Pereira et al., 2009). Adding additional ingredients such as fruits, nuts or coffee (Felberg et al., 2009) is another way to mask it.

Probiotics such as Lactobacillus and Bifidobacterium spp. are bacterial members of the normal human intestinal flora that exert several beneficial effects on human health and well-being through production of short-chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, risk reduction for colon cancer, immune system stimulation and lowering of serum cholesterol levels (Ranadheera et al., 2010). Probiotics are recognized for their applications in dairy products, particularly yoghurts, and the market for these products is still expanding. However, the development of lactose-free probiotic products is still needed and demanded. Coffee beneficial characteristics to health as well as its strong flavor and good acceptability by consumers in general make of this beverage a good candidate to flavor soy products, increasing is acceptability. Furthermore, studies have reported that coffee exert antimicrobial activity due to its active components such as melanoidins, caffeic acid or trigonelline which have been exploited as natural food preservatives (Mueller et al., 2011).

Considering all benefits of soy and coffee, a dessert containing these two food products and probiotics was developed (Duarte et al., 2014). Due to the antimicrobial effect of coffee, however, it was necessary to certify that coffee would not inhibit the growth and viability of probiotics.

This study aimed to evaluate the effect of instant arabica coffee on the viability of the probiotics *Bifidobacterium bifidum* subsp. *lactis* and *Lactobacillus acidophilus* in a soy-coffee dessert during cold storage.

2. MATERIAL AND METHODS

2.1. Dessert Production

Soymilk powder **(**Provesol SM-N) was provided by Olvebra Industria SA, RS, Brazil; probiotic lactic culture consisting of *Lactobacillus acidophilus* (La-5) and *Bifidobacterium bifidum* subsp. *lactis* (BB-12*)* was from Christian Hansen, SP, Brazil; good quality organic light-medium roasted instant arabica coffee was provided by Native, SP, Brazil; sugar cane

derived sucrose was comercial; aditional mix of stabilizing base ingredients was from Sabor Alternativo (PR, Brazil).

Soymilk powder at 10%, sugar (15%), mix of gums and stabilizer were subjected to pasteurization to reduce the possibility of existing microbial counts and inactivation of thermolabile antinutrient factors (Silva and Silva, 2000). Following, the solution was inoculated with the probiotic starter culture (2% inoculum) at 42°C for 5 h or until pH 4.6 - 4.7. After incubation, instant arabica coffee (Native, SP) was added at concentrations 0%, 0.5%, 1.0% and 1.5% (weight/volume).

2.2. Assessment of Viability of Commercial Probiotic Cultures in the Dessert during storage

The viability of *L. acidophilus* (La-5) and of *B. bifidum* (BB-12) was monitored after the manufacturing process (T0), and during the storage period, which occurred at 7°C (± 1°C) for 21 days. Sample was collected on days 1 7 (T1), 14 (T2) and 21 (T3) after dessert preparation. The formulations were evaluated by counting the microorganisms using direct counting method.

1 g of dessert was blended with 9 mL peptone water at 0.1% and submitted to serial dilutions with the same diluent in duplicates. *L. acidophilus* was counted by pour-plating of each dilution in modified DeMan-Rogosa–Sharpe (MRS) agar, prepared as a basal medium containing maltose (50% v/v), as described by the International Dairy Federation (1995), after 3 days of anaerobic incubation at 37°C. *B. bifidum* (BB-12*)* was monitored by pour-plating dilution in modified DeMan-Rogosa–Sharpe (MRS) agar supplemented with L-cysteine, 0.5 g/l; lithium chloride, 2 g/L; and sodium propionate, 3 g/L after 3 days of anaerobic incubation at 37°C.

The pH was determined using a potentiometer (pHquimis, SP) and titratable acidity (TA) was determined in triplicate of samples according to AOAC (1995) before and during storage, using lactic acid as control.

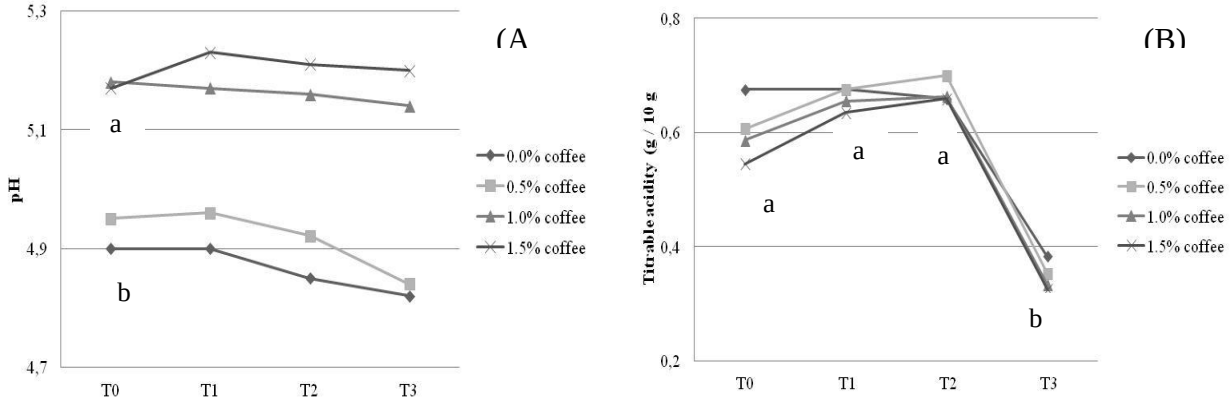
Analyses of variance were carried out to compare storage times using STATISTICA® version 10.0. Differences were considered significant when $p < 0.05$. pH and TA were performed in triplicate. The whole was performed in duplicate.

3. RESULTS AND DISCUSSION

The plain soymilk formulation pH was 4.6 (±0.1) after 5 hours incubation, which is considered ideal for fermented foods (Esteves, 2011). After addition coffee, all formulations had the pH increased from T0.

Figures 1 and 2 show pH and TA values after 0, 7, 14 and 21 storage days at 7 °C. Higher pHs were observed in samples that contained 1.0 and 1.5% coffee, compared to 0% and 0.5% coffee, but this was not reflected in TA. While pH did not change during storage, TA decreased after 14 days storage.

Figure 1. pH (A) and Titratable acidity (B) of soy-coffee probiotic dessert during three weeks storage at 7 °C.



T0 = just after preparation, T1 =7 days storage; T2 = 14 days storage; T3 = 21 days storage.

Tables 1 and 2 show the viability of both cultures (*L.acidophilus* La-5 and *Bifidobacterium bifidudum* BB-12*)* contained in the soy-coffee dessert, immediately after fermentation, and during storage at 7 °C for up to 21 days.

Table 1. Growth rates of *Lactobacillus acidophilus* for different percentages of instant coffee during storage for 2 days at 7 °C.

Instant coffee concentration	*L. acidophilus* La-5			
	T0 (1th day) CFU per 100g serving	T1 (7th day) CFU per 100g serving	T2 (14th day) CFU per 100g serving	T3 (21th day) CFU per 100g serving
0%	9.4×10^{10}	7.9×10^{10}	4.0×10^{9}	8.5×10^{9}
0.5%		6.9×10^{9}	5.4×10^{9}	1.3×10^{9}
1.0%		3.3×10^{9}	2.8×10^{9}	4.6×10^{8}
1.5%		6.6×10^{9}	4.3×10^{8}	7.0×10^{8}

Table 2. Growth rates of *Bifidobacterium bifidum* for different percentages of instant coffee during storage at 7 °C .

Instant coffee concentration	*Bifidobacterium bifidum* BB-12			
	T0 (1th day) CFU per 100 g serving	T1 (7th day) CFU per 100 g serving	T2 (14th day) CFU per 100 g serving	T3 (21th day) CFU per 100 g serving
0%	2×10^{8}	2.9×10^{8}	3.2×10^{7}	3×10^{6}
0.5%		3.9×10^{8}	8×10^{6}	$\leq 10^{5}$
1.0%		1×10^{8}	5×10^{6}	
1.5%		2×10^{8}	1.4×10^{6}	

The lactobacilli showed the greatest ability to grow with the yogurt starter in the soy dessert. Storage decreased the count of *Bifidobacterium bifidum* after 14 days, possibly due to an increase in lactic acid production by *Lacobacillus*. Marques (2012) observed that pH directly affected viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in fermented cows' milk during refrigerated storage. However, in the present study *Lactobacillus acidophilus* count was stable up to 21 days storage.

The reasons why the count of *Bifidobacterium bifidum* decreased after 7 days storage should still be investigated. The low water activity caused by high sugar concentration, and the use of stabilizers may have contributed to this fact (Farnworth, 2007).

Considering that the viable counts of probiotic bacteria should not decreased more than 6 log10 CFU/g during storage in order to have sufficient numbers of this microorganisms able to exert the desired therapeutic effects (Cruz et al., 2010), and based on the Brazilian legislation (Brasil, 2002) which have established that in order to be considered probiotic a product must contain at least 10^8-10^9 probiotic bacteria per food serving, the soy-coffee dessert may be considered a probiotic product when served as 100 g serving, as long as it is consumed in a period of up to 7 days after production, when considering *Bifidobacterium bifidum* culture and up to 21 days when considering only *L. acidophilus*.

5. CONCLUSIONS

In the present study, the viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in a fermented lactose-free dessert based on soy and different concentrations of coffee was evaluated. Coffee concentration did not affect the count of both probiotic cultures. Taking in account the Brazilian laws, the evaluated soy-coffee dessert (portion of 100g) may be considered a probiotic product if consumed in a period of up to 7 days after production, when considering *Bifidobacterium bifidum* culture and up to 21 days when considering only *L. acidophilus*.

6. ACKNOWLEDGEMENTS

The authors would like to thank FAPERJ, CNPq, and CBP&DCafé-EMBRAPA (Brazil) for financial support. And they would also like to thank Olvebra Industrial S.A, RS, and Native, SP (Brazil) for supplying food material for this study.

7. REFERÊNCIAS BIBLIOGRÁFICAS

ABIC. Available at http://www.abic.com.br; acessed Apr. 2014.

Cruz, A.G., Walter, E.H.M., Cadena, R.S., Faria, J.A.F., Bolini, H.M.A., Pinheiro, H.P., Sant'Ana, A.S. (2010). Survival analysis methodology to predict the shelf-life of probiotic flavored yogurt. *Food Research International*, 43, 1444-1448.

Duarte, G., Farah, A., Felberg, I., Calado, V., DELIZA, R. Development of soy-coffee fermented product containing probiotics and soybean hull. In: 25th International Conference

on Coffee Science - ASIC, 2014, Colombia. Proceedings of the 25th International Conference on Coffee Science - ASIC, 2014.

Esteves, T.C.F. (2011). *Desenvolvimento de alimento fermentado de soja tipo "iogurte": avaliação da estabilidade física* (Dissertação de Mestrado). Universidade Federal do Rio de Janeiro, Rio de Janeiro.

Farah, A., Donangelo, C.M. (2006). Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology*, 18, 1, 23-26.

Farnworth, E.R., Mainville, I., Desjardins, M.-P., Gardner, N., Fliss, I., Champagne, C. (2007). Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International Journal of Food Microbiology*, 116, 174-181.

Felberg, I., Antoniassi, R., Deliza, R., Freitas, S.C., Modesta, R.C.D. (2009). Soy and Brazil nut beverage: Processing, composition, sensory, and color evaluation. *Ciência e Tecnologia de Alimentos*, 29, 3, 609-619.

International Dairy Federation (IDF). (1995). Fermented and non-fermented milk products. Detection and enumeration of Lactobacillus acidophillus Culture media. FIL-IDF Standard 306. Brussels: International Dairy Federation.

Kies, A.K. (2014). Authorised EU health claims related to the management of lactose intolerance: reduced lactose content, dietary lactase supplements and live yoghurt cultures. *Foods, Nutrients and Food Ingredients with Authorised EU Health Claims*, 177-211.

Marques, A.P. (2012). *Desenvolvimento de bebida láctea fermentada à base de soro lácteo e café solúvel com atividade probiótica* (Dissertação de Mestrado). Universidade Federal de Lavras, Minas Gerais.

Muller, U., Sauer, T., Weigel, I., Pichner, R., Pischetsrieder, M.(2011). Identification of H_2O_2 as a major antimicrobial component in coffee. *Food and Function*, 2, 255-272.

Official Methods of Analysis of Association of Official Analytical Chemists (AOAC) (1995). 16a ed. Washington, 1-2.

Yan, L., Spitznagel, E.(2009). Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *American Journal of Clinical Nutrition*, 89, 1155–1163.

Pereira, M.O., Bampi, M., Rodrigues, F.T., Santa, O.R.D., Santa, H.S.D., Rigo, M. (2009). Elaboração de uma bebida probiótica fermentada a partir de extrato hidrossolúvel de soja com sabor de frutas. *Revista do Setor de Ciências Agrárias e Ambientais*, 5, 3, 475-487.

Ranadheera, R.D.C.S., Baines, S.K., Adams, M.C. (2010). Importance of food in probiotic efficacy. *Food Research International*, 43, 1-7.