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EFFoST
European Federation of Food Science and Technology

29th EFFoST Conference

Food Science Research and Innovation: Delivering sustainable solutions to the global economy and society

10-12 November 2015 | Athens, Greece

CONFERENCE PROCEEDINGS-VOLUME I

www.effostconference.com

Edited by:

Dr. Efimia Dermesonlouoglou, Dr. Virginia Giannou, Dr. Eleni Gogou & Prof. P. Taoukis
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ISBN: 978-618-82196-1-8

CONTENTS

Production of synbiotic fermented soymilk from vegetable soybean

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ABSTRACT

Vegetable soybeans [*Glycine Max* (L.) *Merril*] are harvested while seeds are immature which have some advantages over mature soybean, such as sweeter flavor and less content of indigestible oligosaccharides, stachyose and raffinose. Considering those beneficial characteristics the aim of this work was to study the fermentation process of soymilk from vegetable soybean and evaluate the influence of prebiotics, inulin and fructooligosaccharide, on physical-chemical and microbiological properties of the fermented beverage.
Soymilk was produced by soaking 50 g of freeze-dried vegetable soybeans cultivar BRS-232 in 455 g of water for 10 minutes. The mixture was heated at 85 °C and blended for 3 minutes. The slurry was stirred for 5 minutes, filtered and the soymilk supplemented with 4 $g{\cdot}ml^{-1}$ fructooligosaccharides (FOS) or inulin, or a mixture of both. Fermented soymilk without the addition of prebiotic was also prepared. The four formulations were produced in triplicate. Soymilks were inoculated with 0.02% of a freeze-dried ABT-4 culture, containing *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* susbsp. *lactis* Bb-12 and *Streptococcus thermophilus*, incubated at 37 °C until pH reached 4.8 and stored at 5 °C for 28 days.
Prebiotics had no effect on time needed to reach pH of 4.8 in soymilk that was obtained in 3.2 ± 0.2 h. Lactic acid production was 15 % higher in fermented soymilk with inulin during storage. Viable cell numbers of *B. animalis* remained above 10^8 cfu/mL in the fermented soymilk during storage while *L. acidophilus* decreased by 1 log cfu/mL in the same period. The content of stachyose and raffinose decreased after fermentation by 28.5% and 39.5 % respectively. Soymilk made with vegetable soybeans has a great potential as a vehicle to deliver probiotics to consumers. The total count of probiotics was higher than 10^{10} cfu in a daily portion of 100 mL of the fermented beverage.

Keywords: Vegetable soybean; Fermented soymilk; Prebiotic; Probiotic

INTRODUCTION

A probiotic fermented soymilk combines the beneficial properties of soy with the health benefits of probiotic microorganisms. The fermentation of soymilk is usually carried out by a mixture of a probiotic culture with a yogurt culture, so the acidification rate is increased and the sensory characteristics of the product is improved (Champagne et al., 2009; Saad et al., 2013; Xiao, 2008). Prebiotic ingredients, such as inulin and fructooligosaccharides, may also improve the activity and survival of probiotic bacteria in fermented soymilk (Bedani et al., 2013; Rinaldoni et al., 2012). Moreover, the combination of prebiotics and probiotics results in a synbiotic effect on gut microbiota (Ramchandran & Shah, 2010).
Vegetable soybean [*Glycine Max* (L.) *Merril*] is a popular food that is consumed in Asia, the United States and other countries, mainly as a snack or in salads. It is a soybean that is harvested while the seeds are at approximately 80 % of maturity, such that it has a green color and a soft texture (Saldivar et al., 2010; Song et al., 2003; Wszelaki et al., 2005). These immature seeds have advantages over mature soybean, including improved sensory attributes and nutritional value, such as sweeter flavor and less contents of stachyose and raffinose, resulting in better digestibility (Huang et al., 2014; McWilliams et al., 2004; Saldivar et al, 2011). Although there are many studies about fermentation of soymilk by probiotic microorganisms, little is known about the behavior of those bacteria in soymilk produced from vegetable soybean.
The aim of this study was to evaluate the fermentation process of soymilk from vegetable soybean by probiotic microorganisms and yogurt culture and evaluate the influence of prebiotics, inulin and fructooligosaccharide, on physical-chemical and microbiological properties of the fermented beverage.

MATERIALS & METHODS

Soymilk, from vegetable soybeans, cultivar BRS232 (Embrapa Soybean, Brazil), was produced by soaking fifty grams of freeze-dried vegetable soybeans in 455 g of water at room temperature for 10 minutes. The mixture was heated at 85 °C and blended for 3 minutes. The slurry was stirred at 85 °C for 5 minutes and filtered in a 0.5-mm conical sieve to obtain the soymilk.

The prebiotics, 4 g·mL^{-1} of fructooligosaccharides (FOS) (Orafti® P 95, Beneo Latinoamericana), 4 g·mL^{-1} of inulin (Orafti®GR, Beneo Latinoamericana) or a mixture of 4 g·ml^{-1} FOS and 4 g·mL^{-1} inulin were added to soymilk and pasteurized at 75°C for 15 seconds. The soymilk supplemented with prebiotic was cooled at 37 °C, inoculated with 0.02% of a freeze-dried ABT-4 culture (Christian Hansen, Denmark) containing *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* susbsp. *lactis* Bb-12 and *Streptococcus thermophilus* and incubated at 37 °C until the pH reached 4.7 – 4.8. The fermented beverages were stored at 5 °C for 28 days. All treatments were replicated three times.

Lactic acid were determined according to Rivas et al. (2012) using an Agilent 1200 series HPLC instrument with a refractive index detector (Agilent, Waldbronn, Germany). The contents of oligosaccharides, stachyose and raffinose, were determinate using a 1100 series Hewlett-Packard chromatograph equipped with a refractive index detector (Rivas et al., 2012).

Viable cells numbers of *Lactobacillus acidophilus*, was determined by surface-plating 20 µL of diluted samples in M-MRS agar (formulated MRS agar containing 20 g·L^{-1} of maltose instead of glucose). Plates were incubated at 37 °C for 48 h under aerobic conditions (Lima et al., 2009). *Bifidobacterium animalis* was monitored by surface-plating 20 µL of each dilution in De Man Rogosa Sharpe (MRS) agar containing 0.2 g·L^{-1} of bile salts, 0.3 g·L^{-1} of sodium propionate, 0.5 g·L^{-1} of cysteine - HCl and 0.2 g·L^{-1} of lithium chloride. Plates were incubated at 37 °C for 48 h under anaerobic conditions (GasPak™, BD BBL™, EUA) (Lima et al., 2009). *Streptococcus thermophilus* were determined by surface-plating 20 µL of each dilution in M17 agar supplemented with 5 g·L^{-1} lactose and plates were incubated at 37 °C for 48 h under aerobic conditions (Richter & Vedamuthu, 2001).

RESULTS & DISCUSSION

The addition of prebiotics, fructooligosaccharides or inulin or their combination to soymilk did not influence the fermentation time. The time needed to reach pH of 4.8 ranged from 3.1 to 3.3 h, with no significant difference ($p > 0.05$) between data (Table 1). All beverages presented a significant ($p < 0.05$) increase in lactic acid content during 28 days of storage. The highest increase in lactic acid content (0.48 g/L) was observed in beverage containing inulin (Table 1). The production of lactic acid can be attributed mainly to *Streptococcus thermophilus* that is a homofermentative species and produces lactic acid to a higher extent than probiotic bacteria in soymilk (Wang et al., 2003).

Table 1. Fermentation time of soymilk and lactic acid content in the fermented beverage after 1 and 28 days of storage at 4 °C. Soymilk with no prebiotic (Control), with Fructooligosaccharide (FOS), with Inulin and with FOS and Inulin.

	Prebiotic			
	Control	FOS	Inulin	FOS + Inulin
Time to reach pH 4.8 (h)	3.33 ± 0.22^a	3.22 ± 0.13^a	3.19 ± 0.10^a	3.13 ± 0.05^a
Lactic acid (1 day)	2.09 ± 0.13^a	1.79 ± 0.04^b	2.11 ± 0.14^a	2.13 ± 0.06^a
Lactic acid (28 days)	2.24 ± 0.03^b	2.28 ± 0.16^b	2.59 ± 0.12^a	2.42 ± 0.07^a

Values are expressed as mean ± Sd. ^{a,b}Different letters in a row denote significant difference between trials ($p < 0.05$).

The addition of FOS and/or inulin in soymilk had no significant ($p > 0.05$) effect on *Streptococcus thermophilus* and *Bifidobacterium animalis* viability in fermented beverages. The *Bifidobacterium animalis* Bb-12 viability in fermented vegetable soymilk was greater than *L. acidophilus* La-5. At the end of 28 days of storage, the viable cell number of *B. animalis* was higher than 8 log cfu·mL^{-1} in all of the fermented beverages, regardless of the presence or absence of prebiotic (Figure 1). The decrease in *Lactobacillus acidophilus* population in fermented soymilk during storage was significant ($p < 0.05$) and higher than 1 log cfu·mL^{-1} for all treatments. In general, beverages with inulin and/or FOS presented significantly ($p < 0.05$) lower counts (approximately 0.2 log cfu·mL^{-1}) compared to beverages without prebiotics (Figure 1).

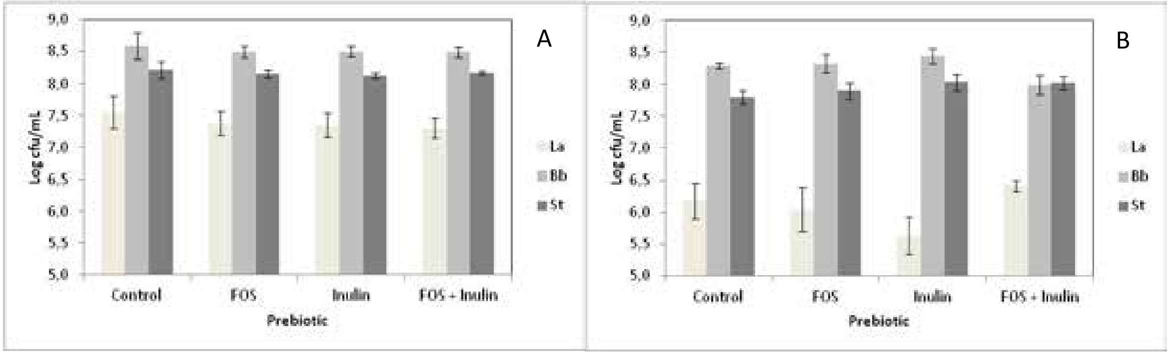

Figure 1. Viable counts of *L. acidophilus* (La), *B. animalis* (Bb) and *S. thermophilus* (St) in fermented soymilk with no prebiotic (Control), Fructooligosacharide (FOS), Inulin and a mixture of FOS and Inulin. A: after 1 day of storage. B: After 28 days of storage.

Oligosaccharides content in soymilk without prebiotic before fermentation were 0.63 g/L and 0.38 g/L of stachyose and raffinose, respectively (Figure 2). After fermentation of soymilk the content of stachyose and raffinose decreased significantly ($p < 0.05$) to 0.48 g/L and 0.22 g/L, respectively. The hydrolysis of these α-galacto-oligosaccharides requires the enzyme α-galactosidase which hydrolyses α-galactosidase bonds (Scalabrini et al., 1998). *Bifidobacterium* spp., *Lactobacillus acidophilus* and *Streptococcus thermophilus* have α-galactosidase activity, however the utilization of these sugars varied with the culture species and strains employed (Farnworth et al., 2007; Wang et al., 2003). Donkor et al. (2007) reported that *S. thermophilus* reduced raffinose in soymilk by 64.5%, whereas stachyose was metabolized by over 40% for most of the strains evaluated. Hou et al. (2000) observed that *Bifidobacteium* reduce raffinose by 39% and stachyose by 65% after 48 h of fermentation of soymilk. In the present study, the reduction of raffinose and stachyose was 41 ± 5 % and 23 ± 2 %, respectively. The short fermentation time in our work may have led to a lower reduction in oligosaccharides content.

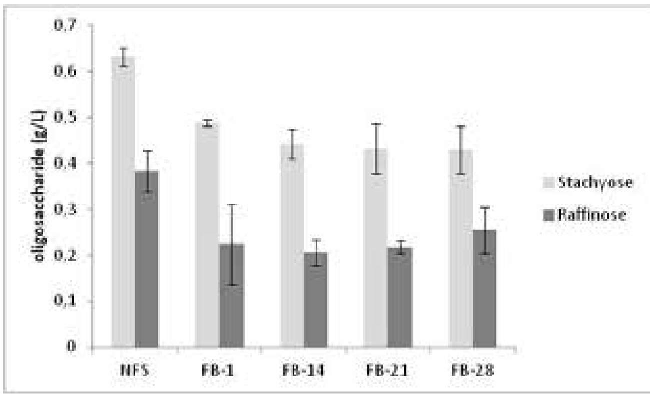

Figure 2. Oligosaccharides content in the soymilk produced with vegetable soybeans and in the fermented beverages (FB) during the storage period. Not fermented soymilk (NFS); FB with 1 day of storage (FB-1); FB with 14 days of storage (FB-14); FB with 21 days of storage (FB-21); FB with 28 days of storage (FB-28).

CONCLUSION

The results demonstrated that fermented soymilk from vegetable soybean is a very promising product as a vehicle for probiotic bacteria, as well as soy product with a lower content of nondigestible oligosaccharides.

ACKNOWLEDGEMENTS

Maua Institute of Technology and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for financial support. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Santander Bank for scholarship.

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