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Enhancement of the antioxidant capacity of soymilk by fermentation with *Lactobacillus rhamnosus*

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ABSTRACT

In order to enhance the bioactivity of soy beverages, fermentation of soymilk was assayed using *Lactobacillus rhamnosus* CRL981 as a β-glucosidase producer. The biological activity of the fermented soy beverage enriched in isoflavone aglycones was determined by several methods to evaluate different antioxidant mechanisms. Fermented soymilk exhibited higher antioxidant activity (71.2 ± 4.0%) determined by β-carotene bleaching method but lower, although significant, DPPH radical scavenging effect (29.5 ± 0.9%) than the unfermented soymilk used as control. The fermented soymilk extracts were able to inhibit the oxidation of DNA induced by Fenton's reagent. The increase of isoflavone aglycone contents during fermentation was a result of β-glucosidase activity towards isoflavone glucosides. This process allowed obtaining a soy beverage with enhanced antioxidant capacity able to contribute to the health and nutritional status improvement of consumers.

1. Introduction

The generation of reactive oxygen species (ROS) such as hydroxyl, peroxyl or superoxide radicals as well as other oxidant species is inevitable in aerobic metabolism of the human body. They are also produced by exogenous factors as light radiation, X-ray exposure, cigarette smoke and environmental pollution, among others. When ROS production in the body exceeds its natural defense mechanism, oxidative stress takes place leading to the damage of tissues and to cell death. Clinical studies reported that ROS are associated with many age related degenerative diseases, cancer, atherosclerosis, stroke, asthma, hyperoxia, heart attack, arthritis, etc. (Cuzzocrea, 2006; Thomas, 2000). Epidemiological studies suggest that the regular consumption of fruits, vegetables and medicinal herbs, reduces the risk of chronic diseases associated with oxidative damage. Thus, regular consumption of antioxidant-rich-foods may help to reduce the deleterious action of ROS and free radicals, and to balance the oxidative stress related to aging process and serious illnesses. This idea drives a growing trend leading to modify eating habits to improve health and nutritional status of consumer (Frei, 2004).

Synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole, have proved to be quite effective in the retardation process of oxidative degeneration. Despite their efficiency, they have unnatural molecular structures which safety is increasingly questioned and turn out to be hazardous to human health (Williams, 1993). Therefore, the importance of taking advantage of antioxidants obtained from natural sources has considerably increased in recent years due to their low cost and availability in large quantity

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as raw material. Polyphenols, widely distributed in plants, have received much attention among researchers (Heim, Tagliaferro, & Bobilya, 2002) because their good radical scavenging activity.

Soybeans are important polyphenol sources in the diet because of their high levels of isoflavones. These are mainly found as glucosides (daidzin and genistin) but also as its corresponding aglycones (daidzein and genistein). Soybean antioxidant properties are ascribed to them (McCue, Horii, & Shetty, 2004). Besides, it is known that the crop genotype and environmental conditions (location and/or crop year) affect both the isoflavone content as their bioavailability in soybean (Wang & Murphy, 1994). Therefore, the antioxidant activity of soybean products will also depend on these factors (Monje et al., 2006). Although undesirably, the 83.9–98.4% of natural isoflavones in soybeans and non-fermented soy food (including soymilk) occur as glucoside-conjugated forms, which are biologically inactive (King & Bignell, 2000). Several studies have showed that is possible to reverse the glucoside/aglycone ratio in soy food through microorganisms with β-glucosidase activity (Chun et al., 2007; Pyo, Lee, & Lee,2005). Marazza, Garro, and Savoy de Giori (2009) indicated that *Lactobacillus rhamnosus* (L.) CRL981, grown in soymilk, completely hydrolyzed the isoflavone glucosides into their aglycone forms at 12 h of incubation.

Antioxidant activity in soy seeds, some traditional Asiatic foods and soy-based beverages without fermentation has been widely studied (Berghofer, Grzeskowiak, Mundigler, Sentall, & Walcak, 1998; Chung, Ji, Canning, Sun, & Zhou, 2010; Murakami, Asakawa, Terao, & Matsushita, 1984; Rau De Almeida Callou, Sadigov, Lajolo, & Genovese, 2010). However, there is very little information available regarding to the antioxidant activity of fermented soymilk (Wang, Yu, & Chou, 2006) and there are not reports about fermented soy products from Argentina.

Many analytical methods for measuring the antioxidant capacity of food extracts have been developed and their merits and disadvantages have been fully discussed in several reviews (Frankel & Meyer, 2000; Roginsky & Lissi, 2005). The consensus is that multiple methods, based upon different reaction mechanisms, should be used. Therefore, the aim of this work was to evaluate the antioxidant capacity of fermented soymilk and to correlate with its enrichment in isoflavone aglycone. The soy beverage was prepared using Argentine soybeans and fermented with *L. rhamnosus* CRL981 as starter culture. Several antioxidant activity methods evaluating different reaction mechanisms were carried out comparing soymilk properties before and after the fermentation process. DNA protection against oxidative damage by fermented soymilk was also demonstrated.

2. Materials and methods

2.1. Microorganism and soymilk fermentation

L. rhamnosus CRL981 belonging to the culture collection of Centro de Referencia para Lactobacilos (CERELA-CONICET) was used as starter for the production of fermented soymilk. Previously, the microorganism was propagated (2%, v/v) twice in MRS broth (De Man, Rogosa, & Sharpe, 1960) and incubated at 37 °C for 18 h without stirring in microaerophilic condition.

Fermented soymilk was prepared from Argentine soybeans through a fermentation process with *L. rhamnosus* CRL981 as was previously described (Marazza et al., 2009). Whole soybeans were washed and soaked overnight in distilled water. The swollen soybeans were manually dehulled and ground with water. An amount of 200 g of soybeans was grinded in 1 l water (ratio 1:5 w/v). The slurry was cooked at 80 °C for 15 min, and then filtered through a double layer cheese cloth to separate insoluble residues. The liquid was transferred into glass bottles and sterilized by autoclaving at 121 °C for 15 min. The resulting soymilk was cooled; sucrose (previously sterilized by filtration) was then added to a final concentration of 1% (v/v) and stored at 4 °C before use. Five hundred milliliters of soymilk were inoculated with 4% (v/v) of an active culture of *L. rhamnosus* CRL981, previously transferred in soymilk, and allowed to ferment at 37 °C for 24 h (optimized growth conditions to achieve high isoflavone bioconversion). Samples were aseptically withdrawn at 0, 3, 6, 9, 12 and 24 h, immediately cooled on ice and stored in freezer. Non-inoculated soymilk incubated under the same experimental conditions was used as a control. For isoflavone extraction, samples of fermented and non-fermented soymilk were freeze-dried and stored at −20 °C until used.

2.2. Preparation and quantification of isoflavone extracts (IE)

Isoflavone extraction including β-glucosides and aglycones from fermented and non-fermented soymilk were carried out according to modified method of Lee et al. (2004). Briefly, 250 mg of each freeze-dried samples were extracted with 100 mM HCl (1 ml), acetonitrile (3.5 ml), and bidistilled water (1.5 ml) shaking for 2 h at room temperature. The insoluble residue was separated by centrifugation (10,000*g*, 5 min, 4 °C), and the supernatant was then filtered with a syringe filter (0.45 μm PVDF membrane, Millipore®, Bedford, MA, USA). The supernatants namely isoflavone extracts (IE) were stored at −20 °C for quantification by HPLC and bioactivity assays (antioxidant capacity).

Quantification of isoflavones by reverse-phase high-performance liquid chromatography (RP-HPLC) was performed according to the procedures described by Marazza et al. (2009). RP-HPLC analysis was performed with KNAUER Smartline System (Berlin, Germany), using Pursuit XRs C18 column (150 mm × 4.6 mm, Varian Inc., Lake Forest, CA, USA) and Smartline multiwavelength UV detector. The mobile phase was composed of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in acetonitrile (solvent B). The solvents flow rate was 0.8 ml min^{-1}, using a gradient of 80% A (20% B) at 0 min, steady for 2.5 min 80% A, decreasing to 70% A for 7.5 min, 65% A for 10 min, 60% A for 5 min, steady at 60% A for 12 min, decreasing to 15% A for 7 min, steady at 15% A for 3 min and then increasing to 80% A for 2 min and steady at 80% for A until completing the gradient program of 60 min. Samples were monitored from 254 to 262 nm and area responses were integrated. Glicitein (16 mg l^{-1}) was used as

internal standard (the soymilk used in this study does not contain this glucoside). Isoflavones were identified by retention time and multiwavelength UV spectrums were compared with those of standards. Standards of glucosides (daidzin and genistin) and aglycones (daidzein and genistein) were obtained from Sigma. Results are expressed as mg of flavonoid 100 g^{-1} dry soymilk to correct from concentration variations due to water evaporation.

2.3. Determination of antioxidant capacity

2.3.1. Antiradical activity determination

Free radical scavenging ability was determined using a synthetic 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH· is a stable free radical that shows its maximum absorption in methanol at 517 nm. In the presence of a hydrogen atom donor substance such as a phenolic compound, the absorbance is reduced in a dose-dependent manner indicating the radical depletion (Wu, Chang, & Schiau, 2003). Based on this principle, the antioxidant activity of a substance can be expressed as its ability to scavenge the DPPH radical. DPPH radical was dissolved in methanol to obtain an absorbance equal to 1.00 at 517 nm. All the solutions were daily prepared before use. Aliquots of 300 μl of every IE were added to 3 ml of DPPH solution. The absorbance decrease at 517 nm was monitored spectrophotometrically in cycles of 30 s for 10 min. The antiradical activity percentage (%ARA) of the samples was calculated according to the equation described by Burda and Oleszek (2001):

$$\%\mathrm{ARA} = 100 \times \left(1 - \frac{A_{SS}}{A_0}\right)$$

where A_{SS} is the absorbance of the solution in the steady state and A_0 is the absorbance of DPPH solution before adding the antioxidant. All determinations were performed in triplicate. The absorbance of the system in a steady state was estimated by the mathematical fitting of kinetic curves performed with Origin 7.0 software.

2.3.2. β-Carotene bleaching assay

This assay was carried out according to the method described by Chaillou and Nazareno (2006) with some modifications. Briefly, an aliquot of 500 μl of a saturated β-carotene solution dissolved in chloroform was pipetted into a 50 ml flask containing the same volume of Tween 20. The mixture was evaporated to remove chloroform using a nitrogen stream for 20 min. The final solution was obtained by adding borate buffer pH 9 to absorbance of 1.30 at 460 nm. Linoleic acid solution was prepared by mixing 50 μl of this compound and 500 μl of 100% Tween 20. The mixture was diluted with borate buffer (pH 9) up to 10 ml. Lipoxygenase (LOX) solution was obtained by dissolving 10 mg of the enzyme in borate buffer (pH 9) up to a 10 ml solution.

For the control assay, 2.0 ml of the β-carotene solution and 200 μl of linoleic acid solution were mixed in a 3 ml cuvette. Finally, 200 μl of LOX solution were added to initiate the reaction, which was measured by monitoring the absorbance at 460 nm in cycles of 30 s for 10 min. For antioxidant activity determinations of IE, the previous procedure was performed by adding an aliquot of 300 μl of every sample. All assays were carried out in triplicate at room temperature (25±1 °C). Antioxidant activity (AOA) is calculated as suggested by Burda and Oleszek (2001) as the percentage of inhibition of the β-carotene bleaching of the samples compared to that of the control.

$$\%\mathrm{AOA} = 100 \times \left(1 - \frac{A_S^0 - A_S^\infty}{A_C^0 - A_C^\infty}\right)$$

where A_S^0 and A_C^0 are the absorbance values measured at the initial incubation time for the samples and control, respectively and A_C^∞ and A_S^∞ are the absorbance values measured of the control and the sample, respectively, in the steady state.

The absorbance of the system in the steady state was estimated by mathematical fitting of kinetic curves performed with Origin 7.0 software.

2.3.3. Reducing power

The reducing power of IE was determined according to the method of Oyaizu (1986) with some modifications. An aliquot of 0.2 ml of IE sample was mixed with 0.2 ml of pH 6.6 phosphate buffer (0.2 M) and 0.2 ml of 1% potassium ferric cyanide. The mixture was incubated at 50 °C for 20 min and then 10% trichloroacetic acid (0.2 ml) was added. The mixture was centrifuged at 15,900*g* at 4 °C for 10 min. The upper layer (0.5 ml) was mixed with 0.1% ferric chloride (0.1 ml) and deionized water (0.5 ml). After 10 min of mixing, the absorbance was measured at 700 nm. A higher absorbance of the reaction mixture indicates a higher reducing power. The values were expressed as reducing power percentage (%).

2.3.4. Assay for oxidative DNA strand breaks

Induction of DNA scission by Fenton's reagent was measured using a small plasmid DNA (p29Kat 232), which was isolated by fast plasmid kit (AccuPred® Plasmid Mini Extraction Kit, Bionner), according to the method described by Lee, Kim, Kim, and Jang (2002) with some modification. Briefly, 10 μl of each IE and 0.5 μl of plasmid DNA in 1x TE buffer (10 mM Tris–Cl and 1 mM EDTA) were mixed followed by the addition of 20 μl Fenton's reagent (100 mM H_2O_2, 0.1 mM acetic acid and 1.6 mM $FeCl_3$). The reaction mixture was incubated at 30 °C for 1 h. Plasmid and plasmid treated with Fenton's reagent were used as plasmid control and oxidation reaction control, respectively. After incubation, the samples were analyzed by electrophoresis on 1% agarose gel containing GelRed nucleic acid gel stain at 65 V/20 mA for 45 min. After electrophoresis, the gel was exposed under UV light and the intensity of bands was analyzed using a Quanti Scan densitometer (Biosoft).

2.4. Statistical analysis

All results presented in this paper were the average of three independent assays and reported as means ± standard deviation (SD). Multiple comparison procedure using least significant difference (LSD) was applied to determine which means are significantly different at $p < 0.05$ confidence level. The correlations were established using simple regression and analysis of variance models (ANOVA).

3. Results and discussion

3.1. *Isoflavones bioconversion*

Enzymatic hydrolysis of isoflavone glucosides into aglycones was monitored during soymilk fermentation process. Substrate concentration changes were evaluated and results are shown in Table 1. The total isoflavones concentration, as the sum of daidzin, genistin, daidzein and genistein, in non-fermented soymilk (control) was 145.4 ± 0.5 mg 100 g^{-1} dry soymilk being daidzin and genistin (biologically non-available β-glucoside forms) the major constituents of the flavonoid fraction (70.0%). Aglycone concentration (genistein and daidzein) in fermented soymilk significantly increased with fermentation time reaching a total bioconversion at 12 h of incubation. The final concentration of genistein at 24 h was 122.2 ± 3.6 mg 100 g^{-1} dry soymilk, approximately 1.9-fold higher than daidzein (64.7 ± 3.8 mg 100 g^{-1} dry soymilk). This high level obtained for genistein was expected, due to the high initial genistin concentration in the studied soymilk. Changes of β-glucosidase activity and isoflavones content during fermentation of soymilk with *L. rhamnosus* CRL981 was previously discussed by Marazza et al. (2009). This report indicates a higher efficiency of *L. rhamnosus* to produce β-glucosidase (almost 40 times) than that reported by Chien, Huang, and Chou (2006) in soymilk using a strain of *Streptococcus thermophilus*. In addition, the correlation between β-glucosidase activity and growth at 12 h of soymilk fermentation with *L. rhamnosus* CRL981 was better (1.4 times) than that reported by Donkor and Shah (2008) using lactobacilli as starter.

In our case, this result indicates that it is possible to obtain a fermented isoflavone aglycone-rich soy beverage, containing more genistein than daidzein. It has been well established that the ability of a flavonoid to inhibit free-radical mediated events and chelating activity is directly related to its chemical structure. In this sense, Heim et al. (2002) found that genistein has a higher antioxidant activity than daidzein. Therefore, the high genistein level found in soymilk fermented with *L.*

Table 1 – Changes in isoflavone (IS) concentrations (mg 100 g^{-1}) during soymilk fermentation by *L. rhamnosus* CRL981 at 37 °C.

Isoflavone isomers	Fermentation Time (h)					
	0	3	6	9	12	24
IS-Glucoside						
Daidzin	43.2 ± 8.2a	41.6 ± 4.5a	20.7 ± 2.6b	6.1 ± 0.9c	0.0 ± 0.0c	0.0 ± 0.0c
Genistin	55.5 ± 9.1a	56.1 ± 2.7a	20.7 ± 3.5b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
Total IS-glucoside	98.8 ± 8.1a	97.7 ± 3.5a	41.4 ± 2.8b	6.1 ± 0.9c	0.0 ± 0.0c	0.0 ± 0.0c
% hydrolysis	0.0	1.2	58.4	93.9	100.0	100.0
IS-Aglycone						
Daidzein	23.4 ± 5.3c	22.1 ± 4.2c	35.5 ± 1.9b	61.6 ± 0.9a	63.6 ± 2.5a	64.7 ± 3.8a
Genistein	21.6 ± 3.5d	24.6 ± 4.2d	58.6 ± 2.8c	91.0 ± 4.1b	97.0 ± 7.2b	122.2 ± 3.6a
Total IS-aglycone	45.0 ± 4.3e	46.7 ± 3.2e	94.1 ± 1.2d	152.6 ± 0.9c	160.0 ± 1.5b	186.9 ± 1.9a
Total IS	143.8 ± 9.1c,d	144.4 ± 8.5c,d	135.5 ± 4.3d	158.7 ± 1.5b	160.0 ± 1.5b	186.9 ± 1.9a

Values in each row having the same letter are not significantly different ($p > 0.05$).

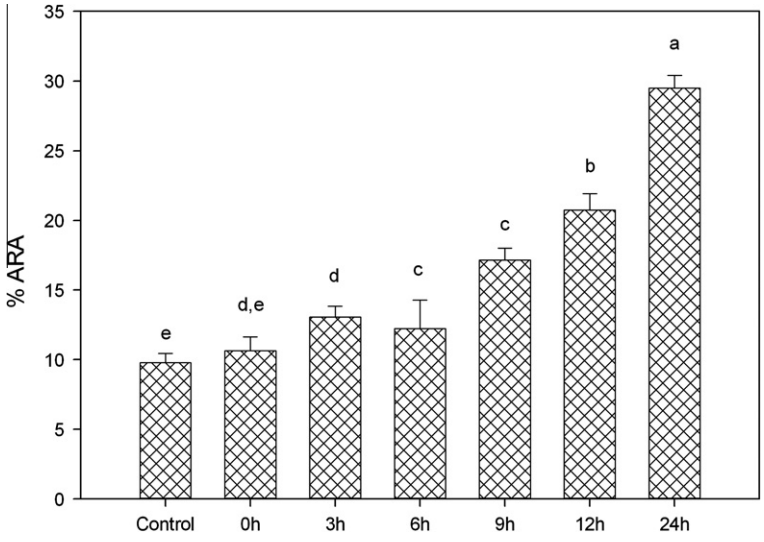

Fig. 1 – Antiradical activity (%ARA) of isoflavone extracts from soymilk fermented with *L. rhamnosus* CRL981 for 24 h at 37 °C. Bars with different letters have significantly different ($p < 0.05$) mean values. The letter 'a' represents the highest value.

rhamnosus CRL 981 may be beneficial to design new functional foods considering preventive and therapeutic nutritional strategies.

3.2. *Antioxidant capacity*

Different antioxidant compounds may act through distinct mechanisms against oxidizing agents; consequently, one isolated method cannot be considered conclusive to fully evaluate the antioxidant capacity of complex foods (Pellegrini et al., 2003). For this reason, four antioxidant capacity assays with different approaches and mechanisms have been carried out.

3.2.1. *Antiradical activity determination*

The antioxidant activity of isoflavone extracts from fermented soymilk samples, determined by the DPPH radical scavenging, is shown in Fig. 1. The ARA (%) slowly increased through fermentation process, being this activity high even initially compared with the control (isoflavone extracts from un-inoculated soymilk incubated under the same assay conditions). However, a more significant activity increase occurred after 9 h of fermentation reaching a 29.5% at the end of fermentation process (24 h). This result indicates that the isoflavone aglycones formed by fermentation are more active antiradical compounds that their β-glucoside precursors. Daidzein and genistein, generated by the action of β-glucosidase enzyme from *L. rhamnosus* CRL981 can act as better hydrogen donors to effectively scavenge DPPH radical (Tachakittirungrod & Okonogi, 2006). This fact would explain the higher antiradical activity (three times) observed in the fermented soymilk with respect to the control (un-inoculated soymilk). By contrast, Lee et al. (2005) have indicated that

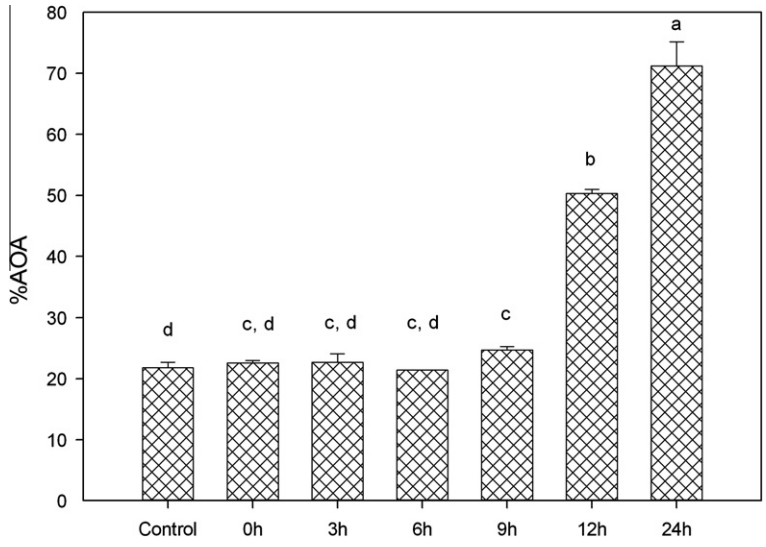

Fig. 2 – Antioxidant activity (%AOA) of isoflavone extracts from soymilk fermented with *L. rhamnosus* CRL981 for 24 h at 37 °C. Bars with different letters have significantly different ($p < 0.05$) mean values. The letter 'a' represents the highest value.

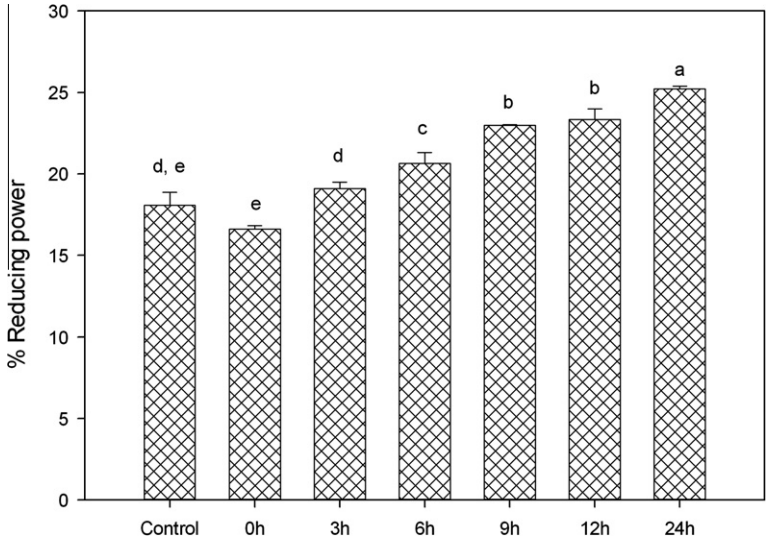

Fig. 3 – Reducing power (%) of isoflavone extracts from fermented soymilk samples (soymilk inoculated with *L. rhamnosus* CRL981 at 37 °C after different incubation times). Bars with different letters have significantly different ($p < 0.05$) mean values. The letter 'a' represents the highest value.

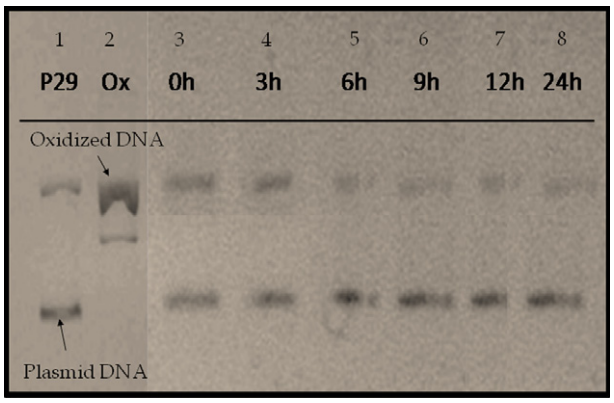

Fig. 4 – Effect to isoflavone extracts from fermented soymilk samples on plasmid DNA, strand breaks induced by Fenton's reagent. Line 1: plasmid DNA control; line 2: oxidized DNA; line 3–9: plasmid DNA with fermented soymilk samples.

daidzein and genistein did not strongly scavenge DPPH radicals.

3.2.2. *β-Carotene bleaching assay*

The antioxidant activity (AOA) of fermented soymilk samples remained constant during the first 9 h of incubation with a mean value of 22.6 ± 0.4% (Fig. 2), results that could be ascribed to the presence of isoflavones which constitute the main polyphenols in soybean (Persky & van Horn, 1995). However the maximum value of AOA (71.2 ± 4.0%), reached at 24 h of fermentation, was attributed to the amount of aglycones released from glucosides by the action of β-glucosidase enzyme of *L. rhamnosus* CRL981.

The antioxidant activity of isoflavone extracts from fermented soymilk is comparable with other polyphenolic compounds (i.e. quercetin, rutin, naringenin, α-tocopherol) widespread in plants. These compounds have the ability of inhibiting lipid autoxidation reactions and scavenging of free radicals (Rice-Evans, 1999). Burda and Oleszek (2001) evaluated the antioxidant activity of several flavonoids and found that synthetic antioxidant 2,6-bis(1,1-dimethylethyl-4-methylphenol) (BHT) and α-tocopherol were effective antioxidants. In our study, the fermented soymilk antioxidant activity was lower (71.2 ± 4.0%) than that of BHT and α-tocopherol (95.3% and 95.8 %, respectively), but was higher than for other flavonoids as quercetin, a highly active aglycon produced by enzymatic hydrolysis of quercetin-3-O-rhamnoglucoside (Burda & Oleszek, 2001).

Many researchers have reported the antioxidant activity of isoflavones and other phenolic compounds isolated from whole soybeans or used in pure state. Recently Singh, Singh, Singh, and Nautiyal (2010) working with fungi on solid state fermentation of soybeans showed similar values in AOA after 7 days of fermentation. By contrast, soy milk inoculated with *L. rhamnosus* CRL981 is a micro-heterogeneous complex matrix where the interactions among components define the antioxidant capacity of the fermented product, and a significant evolution of the AOA during fermentation times was found. The results obtained clearly demonstrate that is possible to enhance the antioxidant activity of soymilk through fermentation process with lactic acid bacteria able to convert isoflavones.

3.2.3. *Reducing power*

Fermented soymilk reducing power was higher (1.5-fold) than those of the control reaching the highest value (25.2%) at 24 h of fermentation (Fig. 3). It has been observed that the reducing power was closely related with the antioxidant activity of polyphenolic compounds (Yen, Duh, & Chuang, 2000). The mean total phenolic compounds (TPC) of fermented soymilk were 94.8 μg of gallic acid/ml of IE (data not shown) and remains constant along fermentation process. Isoflavone aglycones are the major polyphenols presents in fermented soymilk and are responsible for the increase found in the reducing capacity.

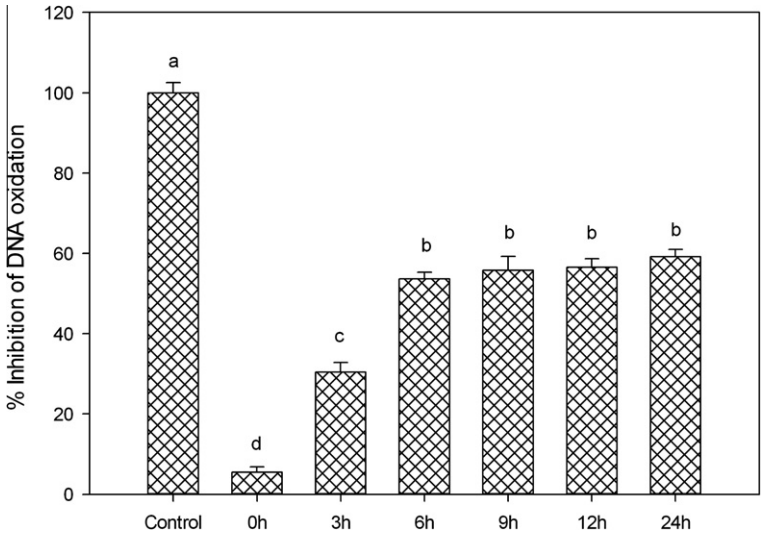

Fig. 5 – Protective effect of isoflavone extracts from fermented soymilk samples upon DNA oxidation induced by Fenton's reagent. The density of the band was quantified using a Quanti Scan densitometer (Biosoft). Bars with different letters have significantly different ($p < 0.05$) mean values. The letter 'a' represents the highest value.

3.2.4. Oxidative DNA strand breaks

Isoflavone extracts of soymilk samples were able to inhibit the oxidation of DNA induced by Fenton's reagent (Figs. 4 and 5). This ability increased along fermentation time reaching the maximum value of 59.2 ± 1.8% at 24 h. This increment is ascribed to the higher concentration of aglycones which have free phenolic groups in their structures able to scavenge hydroxyl radicals generated by Fenton's reaction (Cadet et al., 2002). Other authors have determined the ability of several natural compounds to inhibit the plasmid DNA oxidation (Chandrasekara & Shahidi, 2011; Madhujith & Shahidi, 2009). Singh et al. (2009) observed that inhibition percentage of the aqueous extract of leaf (LE), fruit (FE) and seed (SE) of *Moringa oleifera* were 20.1%, 16.0%, and 13.6 %, respectively. In our study, isoflavone extracts from fermented soymilk showed higher inhibition efficiency than those of different aqueous extract of *M. oleifera*. Even though that in another type of fermentations inhibition of oxidative DNA damage were also found (Singh et al., 2010), these findings are of great interest because this is the first time that it has been reported the ability of fermented isoflavone aglycone-rich soymilk using lactobacilli to inhibit the plasmid DNA oxidation.

4. Conclusions

The consumption of natural and healthy foods is nowadays the major interest of consumers. Soybean products are very important foods due to their numerous nutritional benefits and, their global availability. In this work, the enhancement of the antioxidant activity of a soymilk by fermentation with *L. rhamnosus* CRL981 and the ability to protect DNA oxidation was demonstrated. This is the first report which evaluates the DNA protective ability of fermented soymilk. Therefore, we proposed the strain of *L. rhamnosus* CRL981 as starter culture to obtain a soy beverage rich in isoflavone aglycones with enhanced health-promoting properties.

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