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Improvement of the antioxidant activity of soybean (*Glycine max*.) by biotechnological processing

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Abstract

The aim of this study was to investigate the antioxidant activity of soybean seeds (Glycine max.) processed by germination at 25°C for 4 hours followed by lactic fermentation (24, 48, 72 and 96 hours) into a wheat fermentative extract (containing $0.8x10^8$ CFU/mL and a pH 2.5) with a 1, 3 or 5% saccharose supplementation. The antioxidant activity was evaluated as the reducing power of the alcoholic extracts obtained from processed soybeans.

The experimental data have shown an increasing of antioxidant activity by 2.89 folds in germinated seeds compared with that of unprocessed soybean seeds. Continuing the biotechnological processing of soybean by lactic fermentation it was observed that the antioxidant activity increases with fermentation time and reached maximum after 72 hours of fermentation with 5% saccharose added in the fermentative medium (0.929 mg Fe^{2+}/g d.w.). After 96 hours of fermentation, antioxidant activity decreased for all concentration of saccharose added in the fermentative medium.

The dynamics of the antioxidant activity by germination and fermentation of soybean seeds showed that these processes are appropriate and effective for the improvement of soybeans antioxidant proprieties and they can be applied as biotechnological techniques for food enhancement with natural antioxidants.

Keywords: soybean, germination, lactic fermentation, antioxidant activity, reducing power.

1. Introduction

Free radicals generated by exogenous chemicals or endogenous metabolic processes in food systems may cause oxidative damage by oxidizing biomolecules and result in cell death and tissue damage. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherol and glutathione [10]. When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur, resulting in diseases and accelerating aging [24].

For animals, pathological stress may be found when they are fed in high stress condition or exposed to some chemicals [23]. However, antioxidant supplements or foods containing antioxidants may be used to help the human body and animals to reduce the oxidative damage. Synthetic antioxidants are widely used because they are effective and cheaper than natural types but much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation or to protect the human body from the oxidative damage by free radicals [16].

Oxidative stress has been implicated in numerous chronic diseases such as cancer, heart disease, diabetes, and others. Natural antioxidants, therefore, have been considered as valuable food components in preventing chronic diseases. For instance, phenolic compounds in soybean have been reported to protect LDL oxidation and thereby contribute to prevention of cardiovascular disease [9].

The oxidative stress occurs in cells as a consequence of the physiological processes and interactions with the environment and the antioxidants play an essential role in body protection from this degradation [17].

Phenolic compounds, or polyphenols, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. They can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as tannins. Flavonoids are reported to be the most abundant polyphenols in human diets [15].

The physiological role of antioxidants is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus [5].

Soybean and soybean products, containing various amounts of phenolic compounds have been shown to possess antioxidative ability. Fermented foods represent on average one-third of total food consumption especially fermented soybean that are widespread found in many part of the world as a local food, for example, Tua-nao (Thailand), Natto (Japan), Tempe (Indonesia) and Kinema (India). Those products have been reported on their higher antioxidant activity via microbial fermentation [13] due to the break down of the glycosilated forms and releasing the free aglicon.

Lactic fermentation is an alternative way to improve the phenolic content and antioxidant potential in fermented foods.

In this study, we focused on the evaluation of the influence of biotechnological processing by germination and lactic fermentation on the antioxidant activity of soybean seeds (*Glycine max*.).

2. Materials and methods

Materials

Considering the nutritive and functional potential of soybean seeds and also the inconvenience of its sensorial proprieties in raw consumption, we have focused our research on the possibility of improving these characteristics by biotechnological processing via germination and lactic fermentation.

We have used soybean seeds germinated under controlled conditions and introduced them into a lactic fermentation media represented by a wheat bran extract.

Methods

Germination

Soybean seeds (*Glycine max L*.) were washed with tap water, rinsed twice with distilled water and sterilized by immersing for 10 minutes into a sodium hypochlorite solution 1%. Then the seeds were three times rinsed with distilled water and gently dried at 40°C; ungerminated seeds were kept for analysis.

The washed seeds were soaked in distilled water for 12 hours at 25°C, 1:4 (w:v), then washed and placed on a water-soaked paper filter to germinate for 96 hours at 25°C.

Fermentation

The wheat bran fermentative extract was purchased on the local market; the pH and total viable cell counting was determined before fermentation starts (the medium had pH 2.5 and, $0.8x10^8$ CFU/ml *Lactobacillus).*

Germinated soybean seeds were immersed into the fermentative extract with a 1:3 (w:v) ratio and for the improvement of fermentation 1, 3 or 5% saccharose was added in the medium. The seeds were fermented at 35°C for 24, 48, 72 and 96 hours, then washed and dried gently at 40°C for 12 hours. The dried germinated and fermented soybean seeds were milled (0.4mm) and kept at 4°C for determinations.

In table 1 the experiment plan and the legend of notations are showed.

Table 1. *Experimental plan and legend*

Processing	Conditions		Legend
	Time, hours	Saccharose, %	
Germination, 25°C	96	-	SG
Lactic fermentation, 35°C	24	1, 3, 5	F24-1; F24-3; F24-5
	48	1, 3, 5	F48-1; F48-3; F48-5
	72	1, 3, 5	F72-1; F72-3; F72-5
	96	1, 3, 5	F96-1; F96-3;F96-5

Reducing power

The reducing power of the soybean seeds processed by germination and lactic fermentation was determined by colorimetrical method based on the capacity of the antioxidants to reduce Fe^{3+} to Fe^{2+} and to develop pink colour complexes with o-phenanthroline.

The alcoholic extracts of germinated and fermented soybean seeds were obtained by a modified method (14): 5 g of soybean seeds was milled (0.4mm) then extracted in 25mL methanol and maintained at 55°C for 2 h in a shaking water bath at 100 rpm. After filtering through Whatman No. 1 filter paper, the extracts were lyophilized in a Christ Beta 2-8 LD liophyliser (-86°C/ 0.160 mbar) to 2 ml extract to which were added 5 mL ammonium ferric alum, 5 mL sodium acetate and 5mL o-phenanthroline. A blank sample was done without ammonium ferric alum. Both samples were kept in dark for 1 hour and after that, absorbance was determined at 510nm, compared to blank sample (a higher absorbance indicates a higher reducing power).

Concentration of Fe^{2+} was determined considering the standard scale (figure 1), based on the relation between absorbance and known Fe^{2+} concentration.

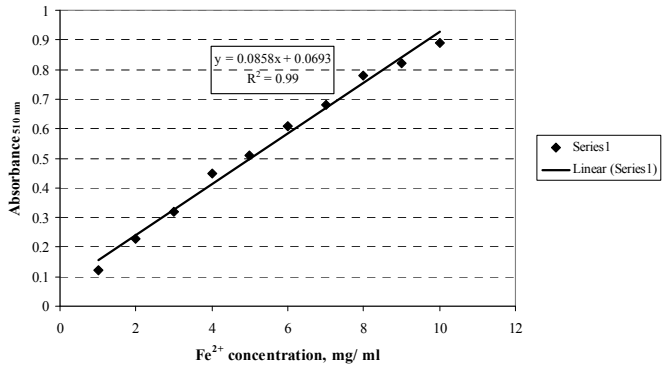


Figure 1. Standard scale for determination of Fe^{2+} level in processed soybeans.

The reducing power was calculated as mg Fe^{2+} / 1g processed soybean seeds (d.w.)

3. Results and discussions

The purpose of this work was to determine the influence of biotechnological processing by germination and lactic fermentation on the antioxidant activity of soybean seeds (*Glycine max.*).

We have investigated the reducing power of soybean seeds germinated at 25°C for 4 days, followed by fermentation at 35°C for 24, 48, 72, 96 hours, by changing the level of saccharose adding in the fermentative medium, to establish if it influences the fermentation process and the reducing power activity in soybean.

The results of experimental work are presented in table 2.

Table 2. Reducing power of soybean seeds processed by germination and fermentation

saccharose level, % / Time, hours	Reducing power, mg Fe^{2+}/ g soybean (d.w.)				
	S	**SG**	**1%**	**3%**	**5%**
24	0,186±0,006	0,537±0,024	0,773±0,009	0,809±0,011	0,821±0,009
48			0,858±0,006	0,878±0,006	0,901±0,011
72			0,877±0,006	0,912±0,011	0,929±0,022
96			0,851±0,010	0,864±0,006	0,897±0,003

* Each value represents mean ± SD (standard deviation) (n=3) and is significantly at $p<0.05$.

In table 1 it can be observed that unprocessed soybean seeds (S) have a good reduction power, 0.186 mg Fe^{2+}/g d.w. but in germinated seeds, this increases by 2.89 times, reaching 0.537 mg Fe^{2+}/g d.w.

The possible mechanism for the improvement of the reducing power of soybean seeds could be related to the changes in izoflavone and their glycosides content by germination. Thus, the activity of β-glycosidase is increased during germination which determines the izoflavone hydrolysis with a consequent release of the aglicon (daidzein and genistein). These are very good antioxidative agents in soybean, protecting the cells from oxidation or cutting the peroxidation chain [11].

Furthermore, the increasing of the phenols level in soybean seeds by germination determines the increasing of the antioxidative proprieties of this seeds, due to their good antioxidant proprieties [12].

Continuing the processing of germinated soybean seeds by lactic fermentation, determines the increase of soybean seeds reducing power and this is depending on fermentation time and level of saccharose supplementation in fermentative medium (figure 2).

It can be observed from figure 2a) that soybean fermentation with 1% added saccharose in the fermentative medium determines the linear increasing of the reducing power from 0.773 mg Fe^{2+}/g d.w. in the first 24 hours of fermentation up to 0.877 mg Fe^{2+}/g d.w. in 72 hours of fermentation.

For the last 24 hours of fermentation, the reducing power of soybean seeds fermented with 1% added saccharose, slightly decreases at 0.851 mg Fe^{2+}/g d.w. (-2.96% compared to those fermented for 72 hours).

The same trend was observed for the reducing power of samples fermented with 3 and 5% added saccharose in the fermentative medium (figure 2 b, c). The maximal reducing

power in these cases was up to 0.912 mg Fe^{2+}/g d.w., for 3% samples and 0.929 mg Fe^{2+}/g d.w. for 5% added saccharose samples.

The decreasing of the reducing power capacity after 96 hours of fermentation was noticed for both concentrations of saccharose 3 and 5%, when the value of reducing power reached 0.864 mg Fe^{2+}/g d.w. and 0.897 mg Fe^{2+}/g d.w. respectively.

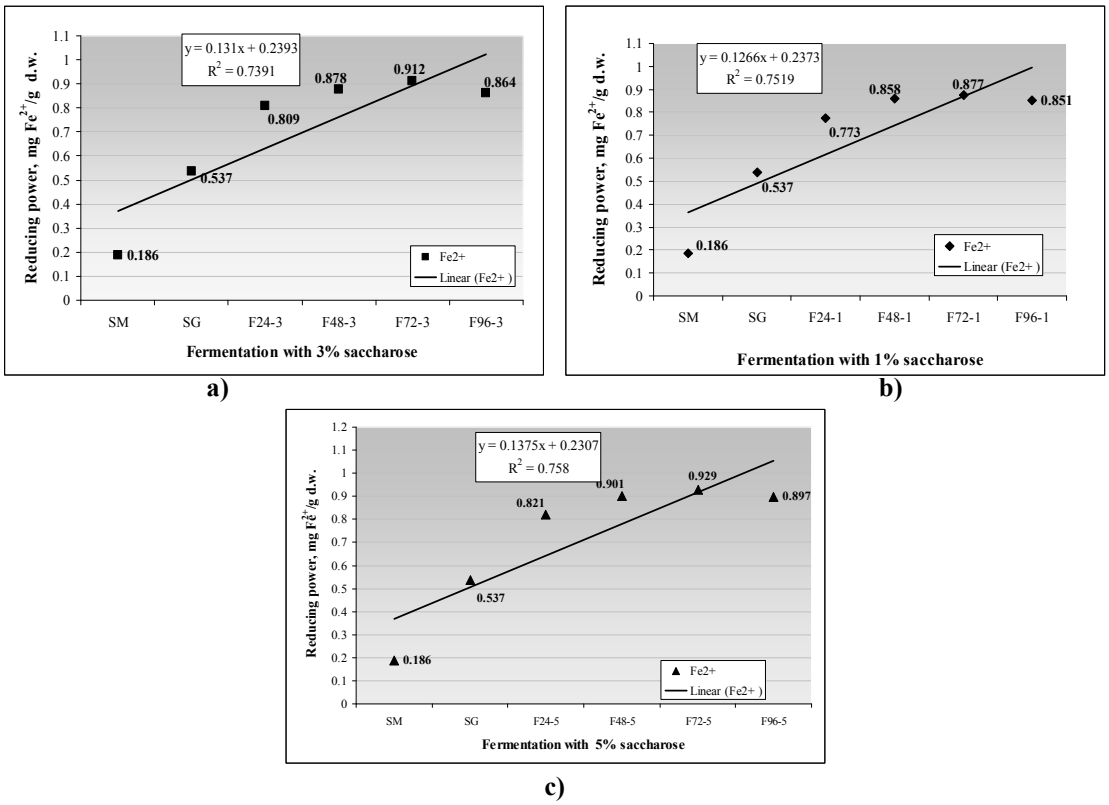


Figure 2. Reducing power of germinated and fermented soybean seeds depending on the saccharose supplementation of fermentative medium: a) 1% ; b) 3%; c) 5%

By analysing the signification and the equations for the reducing power of processed soybean seeds with the level of saccharose supplementation of the fermentative medium (figure 2), it can be concluded that the R^2 (squared Pearson) coefficient is higher than 0.75, and that shows a good definition of the model at a significance level of 5% ($p<0,5$).

Figure 3 is representing the percentage increasing of the reducing power value in germinated and fermented seeds for 72 hours, compared to the value of germinated seeds.

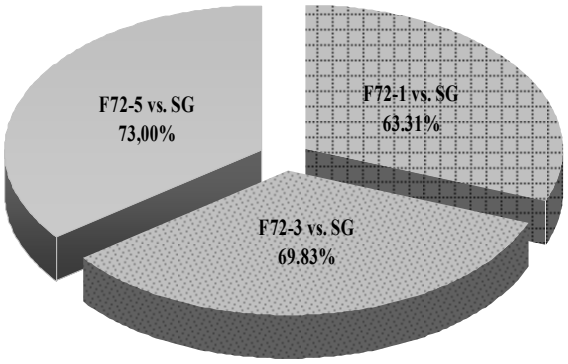


Figure 3. Percentage increasing of the reducing power value in germinated and fermented seeds for 72 hours, compared to the value of germinated seeds

Analyzing the figure 3 it can be concluded that, for the same fermentation time, 72 hours, the increasing of saccharose adding in the fermentative medium determines an improvement of the reducing power value. Thus, the soybean seeds fermented with 5% saccharose registered an increasing of the reducing power with 73.00% compared to the seeds processed only by germination. The percentage of the increasing reducing power for the other two concentrations of saccharose were 63.31% for samples F72-1 and 69.83% for samples F72-3.

The reducing power value has a linear increasing with fermentation time and level of added saccharose, for all fermentation periods (24, 48, 72 and 96 hours) (figure 4).

After 24 hours of fermentation the value of reducing power of soybean seeds fermented with 1% saccharose was with 43.95% higher than that of germinated seeds and reached the value of 0.821 mg Fe^{2+}/g d.w. for those fermented with 5% saccharose.

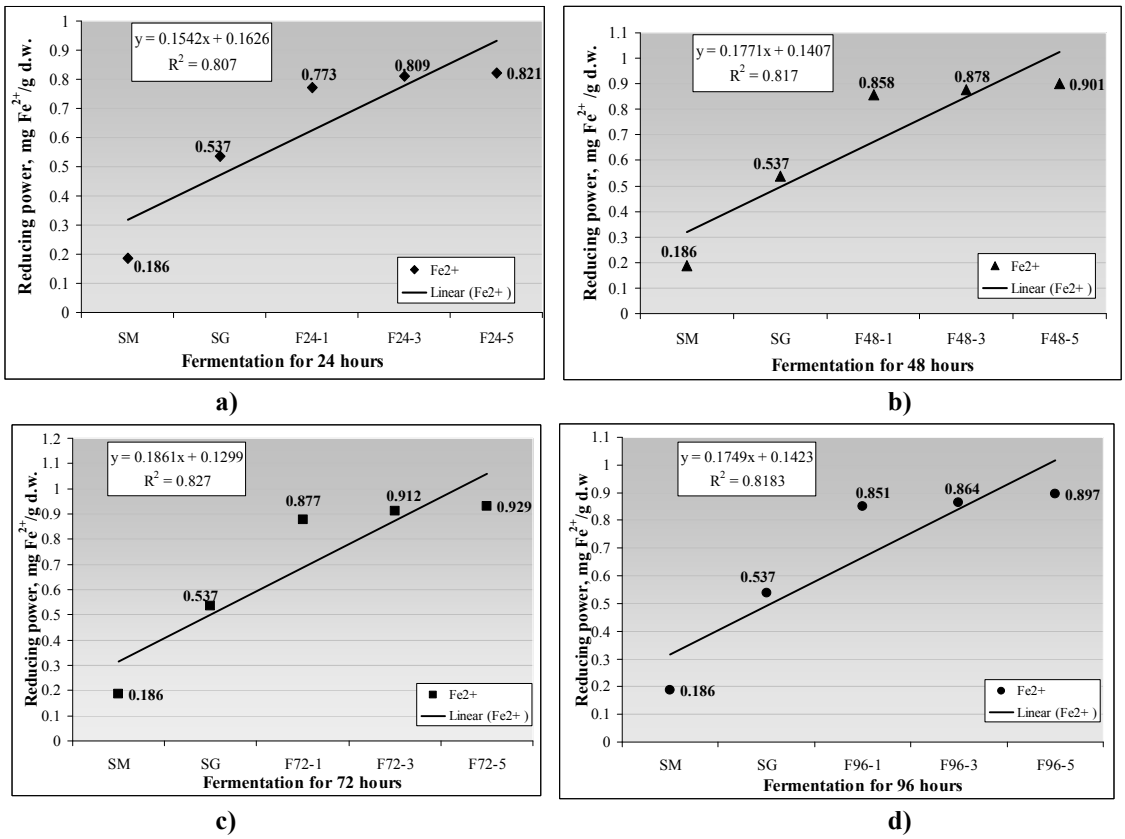


Figure 4. Reducing power of germinated and fermented soybean seeds depending on fermentation time: a) 24 hours; b) 48 hours; c) 72 hours; d) 96 hours

The same increasing trend of the reducing power was observed for all periods of fermentation. The maximum level of reducing power was 0.901 mg Fe^{2+}/g d.w. after 48 hours of fermentation, 0.929 mg Fe^{2+}/g d.w in the third day of fermentation and reached 0.897 mg Fe^{2+}/g d.w at the end of the fermentation (96 hours) (figure 4 b, c, d).

The antioxidant activity is related to the level of isoflavones, total phenols and amino acid content (5, 6). These compounds are known as antioxidative agents and its levels are increasing by fermentation, due to the enzyme activity of lactic bacteria [1].

The decreasing of the reducing power after 96 hours of fermentation can be explained by the slowing of lactic bacteria activity which might determines the decreasing of the phenols levels. The evolution at 96 hours of fermentation could be explained by the reduction of the

level of the amino acids in soybean seeds as they are used by lactic bacteria as a nitrogen source for development; the results are similar with [2].

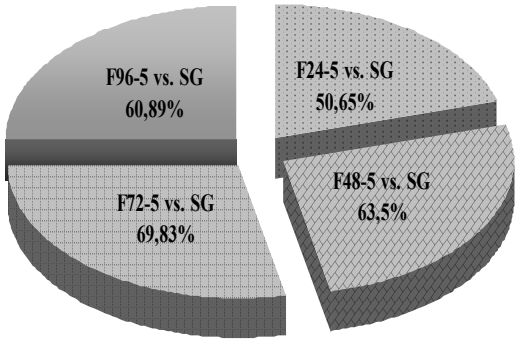
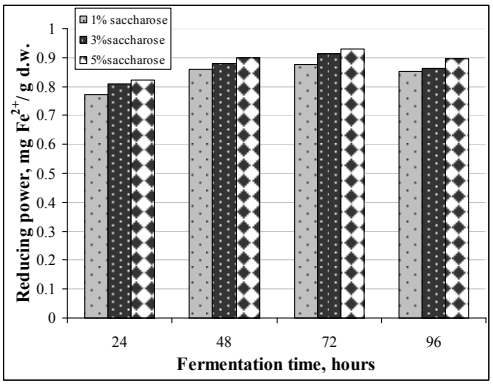


Figure 5. Percentage increasing of the reducing power value in germinated and fermented seeds, compared to the value of germinated seeds

Analyzing the percentage evolution of fully processed soybean seeds reducing power compared to that of germinated seeds (figure 5), it can be observed that this parameter is increasing with fermentation time. The value of the reducing power reached the maximum after 72 hours and is with 69.83% compared to the germinated seeds. The increasing in reducing power obtained after 48 hours of fermentation of soybean seeds is similar with that for 96 hours and higher then those obtained after 24 hours of fermentation.

Figure 6. The evolution of the reducing power of germinated and fermented soybean seeds with fermentation time



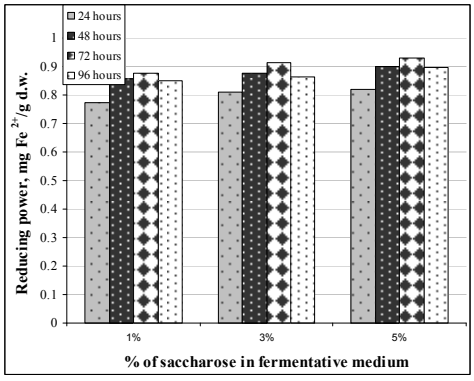


Figure 7. The evolution of the reducing power of germinated and fermented soybean seeds with saccharose supplementation of the fermentative medium

It can be observed in figure 6 and 7 that germination for 4 days at 25°C followed by fermentation at 35°C for 24, 48, 72 and 96 hours with 1, 3 or 5% saccharose added in the fermentative medium improve the antioxidant capacity of soybean seeds.

The biggest increased of the reducing power, as a antioxidant activity measurement, was registered in the seeds germinated and fermented for 72 hours with 5% saccharose, but with minor difference for those seeds fermented the same time period with 3% saccharose.

During tempeh (a soy flour based product) fermentation a partial or total changing of glycoside level occurred; this process was associated with the increasing of the glucosidase and glucoronidase activity, with the synthesis of antioxidant agents in fermented seeds [13].

Another study [16] showed that as a consequence of the microbial protease activity, the level of amino acids is increasing, especially tyrosine, methionine, lysine and tryptophan, known as very good antioxidants.

One possible mechanism for the increasing of the antioxidant activity of soybean seeds processed by germination and fermentation [3]. They observed that the enzymatic hydrolysis of proteins by microbial protease expose the active radicals of amino acids and the peptides resulted from that hydrolysis might exert an antioxidant activity more intense than proteins.

Isoflavones are presented in soybeans in four chemical forms: aglicon, glycoside, acetil-glycoside and malonil-glycoside. Germination and fermentation determine the increasing of free aglicon [7, 19] which are better absorbed in organism [20] and have a very good antioxidant activity, by protecting the LDL cholesterol from oxidation [8] and stopping the peroxidation chain [22].

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