Germinated and Lactic Fermented Soybean Seeds, a Natural Alternative for Healthy Bones. A Scientific Approach

Gratziela Victoria, Carmen Georgeta, Andra Dorina Şuler, Rodica Segal

Abstract
Combining the consumers interests in healthy eating with technology evolution, scientists can increase the food nutritional value by biotechnology. Soybean minerals have lower bioavailability due to phytates, which are hydrolysed by activated phytase during germination. The purpose of this paper is to investigate the relation between soybean seeds germination (4 days at 25°C) and lactic fermentation (24, 48, 72 or 96 hours by immersing soybeans into a medium with Lactobacillus (0.8x10^8 CFU/ml), supplemented with 1, 3 and 5% saccharose). The minerals bioavailability, calculated as mineral extractability in a 0.03M HCl solution. We observed that germination at 25°C for 4 days increased the level of soluble minerals with 48.76% Ca, 22.31% Fe, 15.77% Mg and 28.04% Zn, respectively, compared to control samples. Combining germination with lactic fermentation, the level of soluble minerals increased compared to germinated ones: Ca, 53.4%, Fe, 59.56%, Mg, 43.41% and Zn, 40.87% respectively. Combining germination with lactic fermentation meets the need of consumers for bio, healthy food products offering a good source of minerals. The best processing conditions for obtaining the optimum level of available minerals was germination for 4 days at 25°C followed by fermentation for 72 hours into a lactic bacteria medium containing 3% added saccharose.

Keywords: fermentation, germination, health, minerals, soybeans.

Introduction
Soybean was investigated by nutritionists for a longtime due to its role in preventing or reducing the incidence of heart, artery, bones diseases and even some types of cancer. Alekel (2000) and Chen (2003) proved by clinical trials that a regulate consumption of soybean proteins and isoflavones have increased the mineral bone mass and reduced the risk of osteoporosis. The role of vitamin D (Tucker, 2003, Weaver, 2004), vitamin K (Weber, 2001), magnesium, other micronutrients were also investigated for their involvement in osteoporosis prevention. The low incidence of bone fracture in Japan and eastern countries was correlated with dietary intake, bone health and lifestyle (Ikeda et al., 2006).

Soybean is also a good source of minerals, which availability is reduced by their binding with phytic acid. Germination and fermentation activate the enzyme phytase that releases the minerals from phytates making them available to metabolism.

Considering the findings in the literature, the benefits of soybeans consumption, the "green" processing technologies, we have developed a combined processing method, germination followed by lactic fermentation into a Romanian traditional lactic medium (named bors) in order
to increase the mineral bioavailability in soybean seeds.

**Materials and methods**

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 from the local market, germinated under controlled conditions and introduced them into a lactic fermentation media represented by a wheat bran extract.

**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.

**Minerals determination**

Soluble minerals were determined by their extractability in 0.03N HCl solution (similar to the stomach acid concentration) (Yagoub, 2008): 1g soybean seeds were ground to pass 0.4mm screen and 10mL HCl 0.03N solution was added; the samples were maintained at 37°C for 3 hours under continuous stirring. After HCl extraction, samples were filtered through Whatman 42 filter, dried at 100°C for 1 hour, then burnt in oven at 550°C for 4 hours. Samples were then cooled and 2mL HCl 6N were added, boiled gently for 10 minutes on sand bath, diluted to 100mL with distilled water and minerals were determined by atomic absorption spectrophotometer (FAAS) “Analyst 400” – Perkin Elmer.

Total mineral content was also determined by the same method, without HCl 0.03N extraction. The final results were calculated in ppm (mg metal/kg product) and reported to dry weight.

Extractability was calculated by soluble minerals extracted in HCl 0.03N reported to total mineral amount.

**Results and discussions**

Soybean is a good source of minerals like potassium (1670mg/100 g d.w.), sodium (340mg/100 g d.w.), calcium (350mg/100 g d.w.), magnesium (238mg/100 g d.w.), phosphorus (660mg/100 g d.w.), sulphur (410mg/100 g d.w.), chloride (240mg/100 g d.w.), iodine (0.54mg/100 g d.w.), iron (9.7mg/100 g d.w.) copper (1.29mg/100 g d.w.), manganese (2.8mg/100 g d.w.), zinc (2.2mg/100 g d.w.), aluminium (0.07mg/100 g d.w.).

Bio elements have a very important biological role in cell differentiation process, influence the RNA and DNA synthesis interfering in the growth and development of the body. Meanwhile, they are enzymatic co-factors for important metabolic enzymes.

Intestinal absorption of minerals is possible only if these are in ionic (soluble) forms, thus, identifying the processing methods that increase the availability represent a solution for their nutritional value improvement (Segal et al., 2006).

These mineral’s bioavailability is reduced due to the presence of phytic acid which combines with minerals and forms phytates, insoluble compounds which are not digested by human body due to lack of phytase, the enzyme that splits the complexes and releases the soluble minerals. By germination and lactic fermentation, phytase is activated in soybean seeds, thus valuable nutrients become are available. There are studies (Khalid et al., 2009) showing that the total mineral content do not varies, but minerals solubility increases due to several types of processing (soaking, cooking, germination, sprouting, fermentation) (Bahaciu et al., 2000).
Evolution of soluble calcium (Ca$^{2+}$) by processing

The calcium content of soybean seeds varies with variety, cultivation and harvesting methods and reaches 226-350 mg/100g d.w. (Dikshit et al., 2003).

In this work we have determined the content of soluble calcium in crude, germinated and germinated-fermented soybean seeds, according to the fermentation methodology described above and the results are shown in figure 1.

After 4 days of germination, the soluble calcium level increased with 48.76% compared to crude ones. The Ca-HCl extractability reached 50.58% compared to 34% in unprocessed seeds (figure 1).

The higher are the levels of saccharose in the fermentative medium and fermentation time, the biggest value extractability has (maybe due to intense fermentation and phytase activation). Thus, the soluble calcium of fermented seeds in 5% saccharose medium for 96 hours reached

Table 1. Total minerals in soybean seeds

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total minerals, mg/100 g d.w.</th>
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<tbody>
<tr>
<td></td>
<td>Calcium (Ca)</td>
</tr>
<tr>
<td>Soybean seeds</td>
<td>341.26±0.012</td>
</tr>
</tbody>
</table>

* data represents media of three samples ± standard deviation

Figure 1. Soluble calcium content and Ca-HCl extractability in germinated-fermented soybean seeds
307.63 mg/100g d.w, and the extractability up to 90.16%. These results are quite close to samples fermented in the same amount of saccharose, but less time (72 hours).

Thus, the increasing of the saccharose level in the fermentative medium can determine the reducing of fermentation time with 24 hours: samples germinated and fermented for 72 hours (5% saccharose) have 81.34% Ca-HCl extractability, while those germinated and fermented for 96 hours (3% saccharose) have 86.53% Ca-HCl extractability.

*Evolution of soluble iron (Fe²⁺) by processing*

Although it is found in small amounts in the human body, iron is one of the most important food microelements: it is a compound of haemoglobin, cytochrome, cellular respiration cycle enzymes cofactor. The nutritional value of a food as iron source is related not to the total amount of iron, but to the chemical status of this element, if it is soluble or not (Segal et al., 2006).

Germination is a complex biochemical process, where phytase activation is the most important for mineral bioavailability increasing (Bau et al., 2000). By combined germination and lactic fermentation of soybean seeds, the iron status changed and we investigated in the present paper the evolution of the soluble iron content. Results (figure 2) show an increasing with 22.31% of the soluble iron in germinated soybean seeds compared to those unprocessed. The extractability is also increasing from 44.26% in crude soybeans to 54.12% in germinated seeds. Analyzing the data shown in figure 2, it can be said that combining germination with lactic fermentation determined the improving of the nutritional value of soybean seeds, due to the continuous increasing of soluble iron and Fe-HCl extractability consequently. Thus,
the soluble iron of the germinated and fermented seeds for 96 hours with 3% saccharose content increased with 59.56% compared to germinated soybeans. It is also observed that the level of soluble iron for germinated fermented seeds, 96 hours / 3% saccharose is the same with those processed 72 hours / 5% saccharose. It means that the same benefit (increased soluble iron content) was obtained by reducing the fermentation time with 24 hours and increasing the saccharose level with 2%. This is an important technological finding because a shorter fermentation period reduces the risk of altering the product by different microorganisms, reduced costs for production, shorter production time.

Evolution of soluble magnesium (Mg²⁺) by processing

Magnesium is an activator for many enzymes, especially those transferring phosphorus, in muscle contraction particularly being a modulator of neuromuscular excitability; it also plays an important role in nucleic acid synthesis and some metabolic paths.

It can be observed in figure 3 that germination for 4 days at 25°C determines the increasing of soluble magnesium in soybeans with 15.77%. The extractability in 0.03N HCl solution of magnesium increased from 50.06% in crude soybean seeds to 57.94% in germinated seeds. The difference is not so obvious as in the case of calcium and iron, so germination alone do not have a good impact on magnesium availability in processed soybean seeds.

Germination and lactic fermentation are biotechnological processes that improve both nutritional and sensorial characteristics of soybean seeds by hydrolysis of macromolecular compounds, synthesis of bioactive molecules, decreasing the level of antinutritional factors as tripsin inhibitors and oligosaccharides (Eltayeb, et al, 2008).

![Figure 3. Magnesium amount and Mg-HCl extractability in germinated-fermented soybean seeds](image-url)
In figure 3 it can also be observed that the level of soluble magnesium in germinated and fermented seeds (72 hours with 3% added saccharose) increased with 43.41% compared to germinated seeds. The degree of improvement of mineral bioavailability must be always correlated with the sensorial gain of the processing. Bahaciu et al. (2009) have shown that the best processing parameters to obtain optimum sensorial improvement were: germination for 4 days at 25°C followed by fermentation for 72 hours with 3-5% saccharose in the fermentation medium. At these parameters, Mg-HCl extractability was 83.11% and 84.71% respectively.

Evolution of soluble zinc (Zn\(^{2+}\)) by processing

Zinc is present in several metal-enzymes involved in digestion, in the metabolism of essential fatty acids. Zinc deprivation affects absorption, DNA and collagen synthesis, immunology and bone health. Zinc is also influencing the learning capacity and hormones activities (Sardesal, 2012).

By germination, the level of soluble zinc increases with 28.04% compared to the level found in control samples. The HCl extractability of zinc increased from 34% in control samples to 50.58% in germinated ones.

The biotechnological processing of soybean seeds determines the increasing of soluble zinc level. The extractability grows suddenly in the first 24 hours of fermentation for all the amount of saccharose reaching a level of 76.28%. Fermentation of soybeans for 72 hours with 5% saccharose increased the soluble zinc level up to 1.93mg/100 d.w. a 40.87% increasing compared to germinated soybean seeds.

Figure 4. Zinc amount and Zn-HCl extractability in germinated-fermented soybean seeds
Conclusions

Legumes play an important role in Eastern diets and can be considered as functional foods because they are good sources of proteins, fibre, micronutrients, biologic active compounds like isoflavones. These are some reasons for legumes to be intensively studied, investigated and processed in order to maximise the health benefits of these seeds.

Nowadays, there is a bio, traditional, ecological trend in nutrition and finding or developing new products that meet consumers need for healthy eating, non-processed or minimum processed products. Germinated and lactic fermented (into a traditional Romanian product) could be a viable offer for this segment of consumers and also a business niche opportunity.

Although germination alone determines an increasing of the nutritive value of soybean seeds, these are not very appreciated by consumers due to the remaining bitter, unpleasant taste and smell of leguminous. But continuing germination with lactic fermentation, a better sensorial quality of seeds is obtained due to the activity of lactic bacteria, which furthermore will have benefits on gut health, not only on bones by increasing mineral bioavailability.

Our experiments have shown that the level of soluble minerals increases after 4 days germination at 25°C followed by 72 hours fermentation with 5% saccharose amount in the fermentative medium: Ca with 53.4%, Fe with 59.56%, Mg with 43.41% and Zn with 40.87% respectively, compared to germinated ones.

References