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Effects of Fermentation, Germination, Roasting and Mono-Screw Extrusion Cooking on the Phytate and Iron Contents of Millet Souna Produced in Senegal (*Pennisetum Glaucum*)

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Abstract

Millet is one of the most widely consumed cereals in Senegal and in terms of the country's grain production volume, it ranks second after rice. It is a high energy cereal but also contains a lot of nutrients such as minerals. However, millet contains anti nutritional factors such as phytates which form insoluble complexes with iron and zinc, thus reducing their bioavailability for the body. During millet processing, artisanal units, unknowingly, use methods that are known to reduce phytic acid levels. That is why, we have chosen three of these methods (germination, fermentation, roasting) and evaluated their effects on phytic acid content of millet. The results of these methods are compared to those of single-screw extrusion cooking. They show that the phytates content of raw millet is 7.56 mg / g. The different applied treatments all give reductions of the phytates contents. Thus, the highest reduction rates are obtained for 72 hours of germination with 62% of reduction of phytates, followed by extrusion cooking at 120° C which is 48.42%. Then, come respectively fermentation at 24 hours and roasting at 120° C which give respectively 44.05%, 34.89% of reduction. Whether wet or thermal methods, the degradation of phytates increases when the treatment time increases or when the temperature increases.

For the iron, the contents pass from 7.56 mg / 100g in the untreated sample to 4.48 mg / 100g after roasting at 120° C.

The fermentation at 24 hours and the extrusion cooking at 120° C respectively come with 7.55 and 6.80 mg / 100, while the germination causes a small increase in the iron content which goes from 7.56 to 8.13 mg / 100gs

Introduction

In Senegal, millet is one of the main cereals cultivated with a production of nearly 891,000 tons during the 2018-2019 crop year [1]. Millet represents 35% of national cereal production, just behind rice. It represents with rice, the basis of food and is generally consumed in the form of dough, porridge, couscous, broken or pancakes, etc. It is a cereal with a protein content between 8 and 12% and helps to satisfy the daily caloric needs of local populations [2]. However, like other cereals, millet contains anti nutritional factors (phytates, tannins, oxalates ...). These form insoluble complexes with minerals and proteins that are generally stable during digestion and therefore not absorbable [3]. This presence of anti nutritional factors is a blockage for the consumer to properly have some nutrients of millet grain. In addition, the presence of phytates (myo-inositol 1, 2, 3, 4, 5, 6-hexakis phosphate) in millet, which is one of the main food crops, does not help to improve the nutritional needs of the populations. In fact, micronutrient deficiencies, in particular iron and zinc, pose a real public health problem today. According to anemia affects 70% of children aged 0 to 5 and 54% of women, while zinc deficiency affects 42.8% of children and 58% of women according to COSFAM [4]. According to child feeding practices are still very inadequate [5]. In fact, among children aged 6 to 23 months, only 17% benefit from food diversification, 32% from a minimum frequency of meals, while 7% benefit from optimal nutrition.

Thus, to improve the bioavailability of nutritional elements, methods of reducing phytate levels can be applied. The main objective of this work is to study the effects of extrusion cooking and three other methods used by artisanal processing units to reduce phytate levels in millet.

Material and Methods

Plant Material

The plant material used in this study is millet souna (*Pennisetum glaucum*) purchased from the Castor market in Dakar. This is the Souna variety grown in most parts of Senegal. This millet was then dry cleaned and sieved. Only grains with a particle size greater than 1.5 mm were used.

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Applied Treatments

Germination

For each of the three times (24, 48 and 72 hours) the germination was carried out in three successive stages: Firstly, for each time, three samples were washed with tap water, and then stirred in a 1% bleach solution for 10 minutes before being rinsed again with tap water;

- In a second step, these washed grains were soaked in water during 20 hours in plastic pots with the ratio m /v of 1/3 (mass of grain / volume of water);

- In a third time, after soaking for 20 hours, the millet grains were drained and then introduced into basins which were covered tightly by plastic bags. Each morning, the grains were re-wetted by rinsing with water and then covered again by the plastic sachets;

- After each germination time, the samples were dried in the sun for 2 hours and then in the shade.

The three times of germination that were carried out, were 24, 48 and 72 hours and started after 20 hours of soaking. The samples obtained are named respectively MG24, MG48 and MG72.

Fermentation

The fermentation was carried out in a traditional way without starters. For its realization, the grains washed with tap water, were soaked for one hour with the same ratio m / v (1/3) to moisten them. Then, after soaking, the grains were well drained and then sealed in plastic pots. For each of the fermentation times of 8, 18 and 24 hours, samples were obtained and are respectively named MF8, MF18 and MF24.

Roasting

The roasting was done in a traditional way with a gas fireplace and an iron pan that serves as a hotplate. The millet grains were thoroughly dry cleaned and placed on an empty skillet that was put on fire. The grains are stirred continuously.

Using a probe thermometer, we followed the evolution of the grain temperature up to 80 °C to start taking samples of roasted beans.

Thus, we took three samples of fifty (50) grams per temperature range of 10 °C between 80 and 120 °C. The samples obtained are named respectively MTO80, MTO90, MTO100, MTO110 and MTO120.

Extrusion Cooking

A quantity of 10 kg of moistened millet grains (moisture 30%) was introduced into the cooker-extruder continuously and at a constant rate. The cooker - extruder used is manufactured by TECHNOCHEM and it is of single screw type whose frequency is 50 Hz with a maximum speed close to 900 rpm and provided with a thermometer for monitoring the evolution of the temperature. The temperature increases regularly until 125 °C. But from 100 °C, we took regular samples of one hundred (100) grams of extruded millet with temperature ranges of 5 °C.

Thus samplings were carried out at temperatures of 100, 115, 120 and 125 °C and are respectively named MC-E100, MC-E115, MC-E120 and MC-E125.

Determination of Phytates and Iron Contents

Determination of Phytates

The samples of millet grains treated by the different methods and three samples of untreated (raw) millet dry-cleaned and calibrated like the treated grains, used as a control, were first finely milled, using a PERTEN mill type 3100.

For each sample, we then calculated the dry matter content from the moisture content determined by Method 2001 11 (4.2.11) AOAC 18th Ed.Rev.2007.

Phytate contents were determined by the method described by Latta and Eskin (1980) and Vaintraub et al. (1988) [6, 7].

Determination of Iron

Iron contents were determined by atomic absorption spectrophotometry after drying and mineralization at 550 °C for 2h. Nitric acid is added to the ash obtained and then evaporated to dryness. The residue is dissolved in hydrochloric acid and this solution is analyzed by Atomic Absorption Spectrophotometer (AAS) by the technique of flame (see instructions IN / CH / 02/00). Each determination was made in triplicate and iron assay is performed on the sample with the best phytate reduction for each of the four methods.

The iron determination was determined for each of the four millet processing methods but only the sample with the highest phytate reduction.

Statistical Analysis

The XLSTAT 6.1.9 software was used. The comparison of the difference in phytate and mineral content between the different samples was determined. A significant difference between the samples was observed for $P < 0.05$.

Results and Discussion

The phytate content of untreated millet grains is estimated at 755.33 mg / 100 g dried matter (DM), or about 7.55 mg / g DM. This content is similar to that found by Lestienne (2004) who found a content of 7.62 mg / g DM [3]. Abdallah et al. (1998) studied the phytate levels of ten millet genotypes in Sudan and found values ranging from 3.5 to 8 mg / g [8]. However, a slightly higher average phytate content was found in maize, millet and sorghum flours by Garcıa-Estepa et al. (1999) [9]. Indeed, in their study they found a phytate content of millet that amounts to 10.64 mg /g. This slight difference with our result could be the determining factor of genetic and environmental factors or cultural practices on phytate contents. Buerkert et al. (1998) showed that the application of phosphorus during the cultivation of millet increased the phytic acid content of grains by 25 to 29% [10].

Effect of fermentation on the phytate content of millet souna

Figure 1 below, shows the effect of fermentation on phytate content of millet grain. This effect is measured for the different fermentation times.

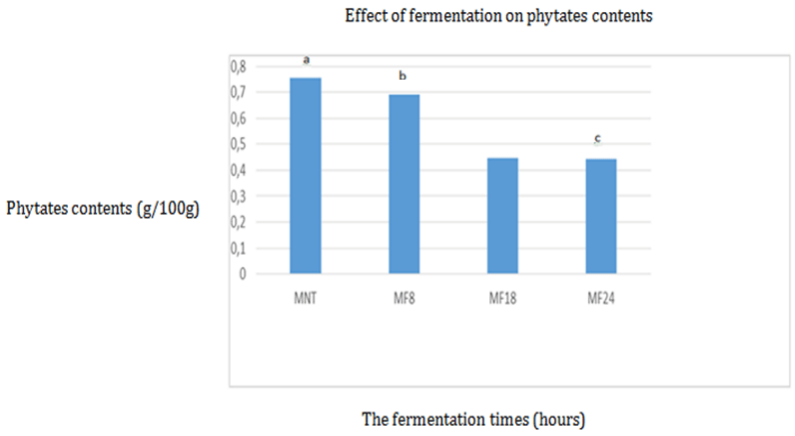

Figure 1: Effect of fermentation on phytates contents of millet

The analysis of Figure 1 shows that for all three times, the fermentation causes significant degradation of phytates. If this reduction (8.44%) for 8 hours of fermentation is relatively low, it is very significant when this fermentation time increases. Thus, fermentations at 18 and 24 hours resulted respectively in reductions of 40.71 and 44.05% of the initial phytates content.

The millet grains fermentation during 12 and 24 hours has been reported to reduce food inhibitors, phytic acid and tannins [11].

These reduction rates obtained for 18 and 24 hours of fermentation are higher than those found by Chitra U et al. (1997) whose fermentation tests led to reductions of phytic acid between 26 and 39% in almost all the studied legumes [12].

The degradation increasing according to the duration of the fermentation could be due to the acidity which reached a pH between 4 and 5 after 24 hours of fermentation. In fact, at this pH, we are witnessing a greater hydrolysis of phytates or a higher enzymatic activity. This pH is close to the optimal pH of S. cerevisae phytase determined in another study (about 4.5) which was also similar to that reported for Citrobacter braakii phytase (pH 4.0) and for E. coli phytase (between 4.0 and 4.5) [13-15].

Fermentation as practiced traditionally and naturally is therefore able to reduce the amount of phytates of millet. It results from the competition of endogenous microorganisms and contaminants that come naturally from raw materials, used equipment and the environment. This complexity of the natural micro flora makes it difficult to control the natural fermentation and can lead to this variability in the phytate content between these different fermentation times [16,17].

Effect of germination on the phytates content of millet souna

In Figure 2, the reduction rates of phytates of millet as a function of the germination time of these grains are presented.

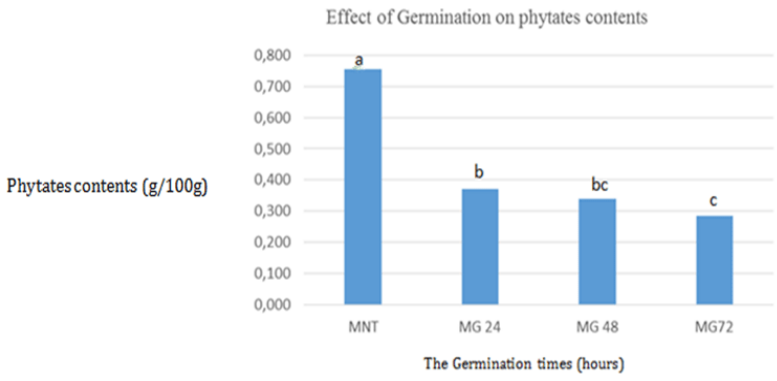

Figure 2: Effect of germination on the phytates contents of millet

Reading Figure 2 shows that phytate reduction is very important with germination. Thus, germination during 24, 48 and 72 hours resulted in phytate reductions of 50.80%, 55.14% and 62.01%, respectively. These phytate reductions are greater than those found by Afify et al (2011) [18]. Indeed, in their study they found reduction rates varying between 24 and 28% for the same germination times.

However, they are close to those found by Khetarpaul and Chauhan (1990) after germination of millet grains at 30 °C for 24 h which reduced phytic acid by more than 50% [19].

Researchers have also reported a decrease in the level of phytic acid during germination due to phytase activity in sprouting grains [20]. Indeed, the activity of phytase was observed during the germination of wheat, barley, rye and oats, allowed to hydrolyze phytates into phosphates and myoinositol phosphates [20]. This remark was also reported by who attributed to germination an activation of the endogenous grain phytase that can degrade phytates [21].

Otherwise, phytates reduction increases with increasing germination time.

Effect of roasting on the phytates content of millet souna

In Figure 3, we see that, the effect of millet roasting on the phytates content. This effect is measured for different roasting temperatures.

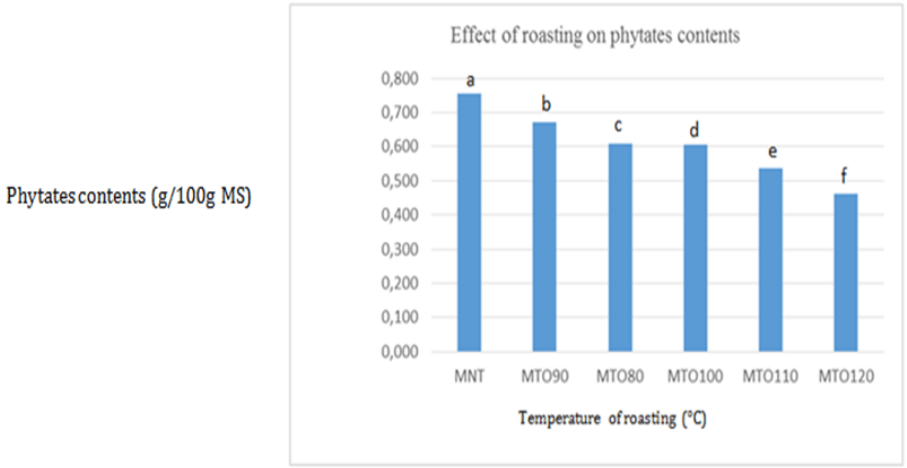

Figure 3: Effect of soaking on phytates contents of millet

The results presented in Figure 3 allow us to see that the roasting of millet has led to significant reductions in phytates that increase with temperature.

Indeed, from 19.31% of reduction of phytates to 80 °C, we reach 34.89% of reduction if the roasting temperature reaches 120 °C. These results show that the rise in temperature causes a degradation of phytates and are confirmed by other authors who had rates of reduction slightly different from ours. Thus, Tiwari et al. (2014) reported that roasting pearl millet grain at 110 °C for 60 seconds reduced the phytic acid content of flour from 7.28 to 4.10 mg / g (43.68%) [22]. Similarly, Chauhan et al. (2015) found that the bursting of pearl millet grains significantly reduced the phytic acid content from 5.164 to 3.738 mg / g (27.61%), which can be attributed to heat treatment according to the authors [23].

Effect of Extrusion Cooking on the Phytates Content of Millet Souna

Figure 4 shows the effect of extrusion cooking of millet grains on phytates contents. This effect is measured for different extrusion temperatures.

Figure 4 shows that extrusion cooking results in phytate reductions ranging between 18.82% at 100 °C and 48.42% when the temperature reaches 125 °C. This is confirmed by other works. The largest reduction achieved during extrusion cooking at 120 °C is slightly less than 54.51% at 140 ° C, found by Satinder Kaur et al. (2013) [24]. Moreover, this result obtained by our study is close to that obtained on samples of African breadfruit flour, corn and defatted soybeans that were extruded in a Brabender single screw extruder (DCE 330, New Jersey). For these samples, extrusion cooking resulted in a 44% reduction in phytic acid content according to [25]. It should also be noted that it is necessary to exceed 100 ° C to have a significant degradation of phytates.

Comparison of the Four Studied Methods

The figure below shows the effect of the different treatments applied to millet souna grains on the phytate content.

Levels with identical letters in column do not have a significant difference ($P < 0.05$).

Figure 5 shows the treatments for which differences in phytate reduction are significant.

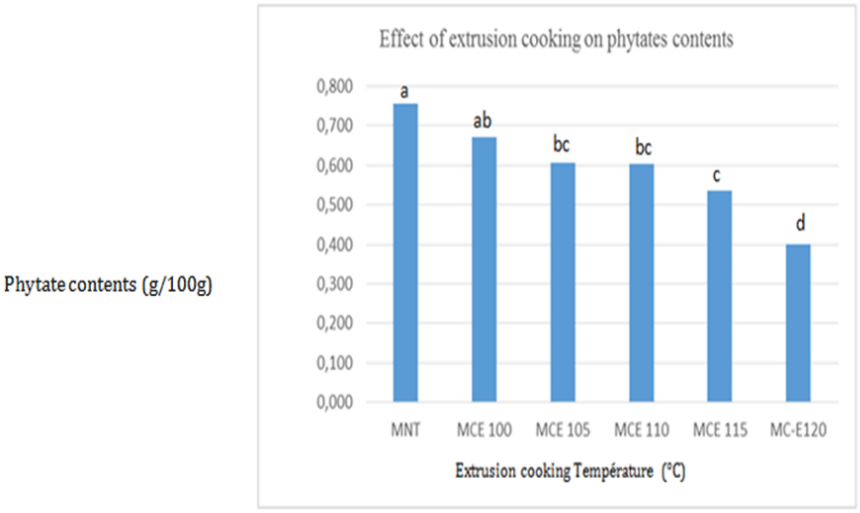

Figure 4: Effect of extrusion cooking on phytates contents of millet

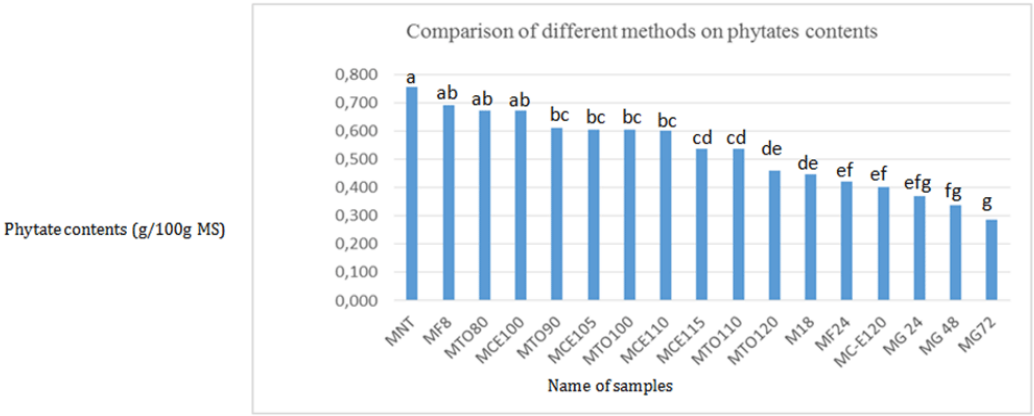

Figure 5: Comparison of effects of different methods on the phytate content of millet souna

In all applied treatments, we notice reductions in the phytate contents of the treated samples. Germination is the method with the best reduction rate, which is around 62.01% after 72 hours. Then, it is around cooking-extrusion at 120 °C which resulted in a decrease of 48.42% of phytates. The 24-hour fermentation and roasting at 120 °C arrive respectively in third and fourth position with, in order, phytate reductions of 44.05% and 34.89%.

Germination gives a better result because in addition to being an effective method for reducing phytates. It could also be considered as a combination of methods because the sprouted samples have been soaked beforehand for 20 hours while for the fermentation, this soaking lasted only one hour in our study. However, according to the results found by Afify et al. (2011) phytate reductions ranging from 23.59 to 32.40% were observed on soaked sorghum grains for 20 hours and from 24.92 to 35.27% for 72 hours of germination [18]. While for extrusion cooking, the sharp decrease from roasting could also be due to the moisture of the sample to be treated. The one that is roasted is dried and has a moisture content of 11% while the extruded sample has been moistened beforehand to a humidity of about 30%. According to Satinder Kaur et al. (2013) who took samples and used the extrusion variables which were moisture content (14, 17 and 20%) and temperatures (115 ° C, 140 ° C, 165 ° C), the most High levels of anti nutrients were observed at 140 ° C and 20% moisture [24].

Comparison of the Effects of the Four Methods on Iron Content

To highlight the comparison between these different treatments, the following figure gives the effect of applied treatments on the iron content.

The iron content of our untreated millet is 7.56 mg / 100g.

This value is close to that found by San-kara Rao and Deosthale (1980) which is 8 mg / 100g but slightly higher than 5.6 mg / 100g found by Nidhi Chaudhary and Swati Vyas (2014) [26, 27].

In Figure 6, we note that for all applied treatments to millet, only roasting leads to a significant reduction in iron content. Thus, this content decreases from 7.56 mg / 100 g in raw millet to 4.48 mg / 100 g after roasting. Then, there is fermentation and extrusion cooking which come respectively with 7.55 and 6.80 mg / 100.

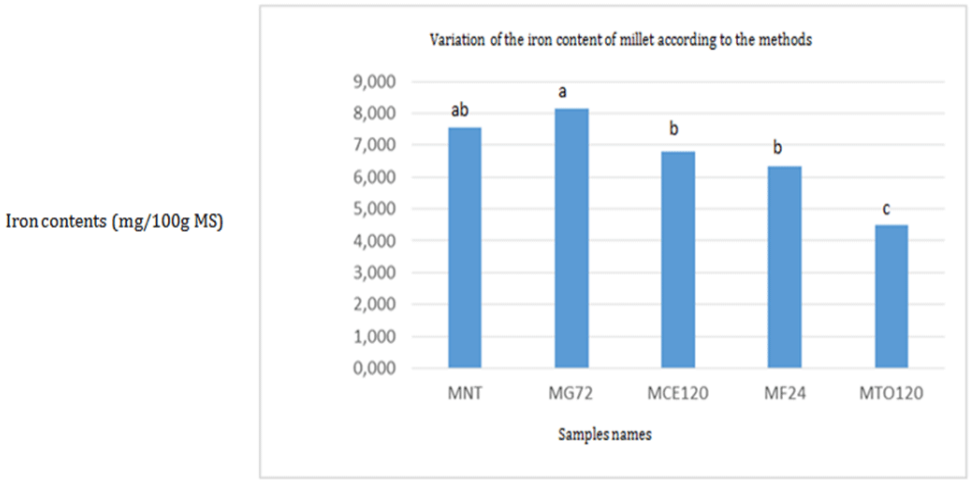

Figure 6: Comparaison des effets des différentes méthodes sur la teneur en Fer du mil

Germination causes a slight increase in the iron content from 7.56 to 8.13 mg / 100g.

According to the iron content of the standard premix was 5.60 mg / 100 g and 7.0 mg / 100 g. At thirty-six and 48 hours, the sprouted premixes had an iron content of 7.6 mg / 100 g and 8.66 mg / 100g. Other authors, such as Tizazu (2010) also found that germination increased the level of minerals, especially calcium, iron and zinc [27, 28].

Between the two thermal methods, we notice a much greater reduction of iron during roasting. This situation could be due to the very short processing time during extrusion cooking (high temperature, short time) unlike roasting.

This significant decrease in iron content during roasting could be explained by the high temperature.

Conclusion

The *Souna* millet variety produced in Senegal has a phytate content comparable to other millet varieties grown elsewhere. The reduction of these phytates can be made possible by using certain traditional processes such as roasting, germination, fermentation but also industrial like extrusion cooking. These different methods all result in a reduction in the phytate content of millet. This degradation of phytates increases with the germination time whereas for thermal methods, it increases as the temperature increases. Of all the methods, germination at 72 hours is the most effective because it leads to a larger phytate reduction with a slight increase in iron content.

On the other hand, the thermal methods compared with each other, give advantage to extrusion cooking at 120 °C which even if it does not lead to a very significant reduction of phytates compared to roasting at 120 °C, but it preserves better the iron.

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