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Improving Nutritional Quality of Cowpea (*Vigna unguiculata*) by Soaking Process

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Abstract Effect of soaking in water and acetic acid solutions to improve nutritional qualities of cowpea was studied. Cowpeas were soaked in tap water, 1 and 2% acetic acid solutions at 4, 8, 16 and 24 hours. Afterwards, phytates and minerals (Fe and Zn) content were quantified. the phytates content of soaked cowpea grain decreased with the acidity of the soaking solution and the duration of treatment. Compared to the raw material, phytates content decrease by 1.48 to 37.59% in samples soaked in tap water at 4 to 24 hours. Likewise, the phytates content decrease by 7.98 to 41.69 in samples soaked in 1 and 2% acetic acid solution at the same soaking time. The best reduction of phytates content (41.69%) is obtained with acetic acid 2% within 24 hours. Regarding minerals, a slight variation with soaking time in their content was obtained. Acetic acid solutions induced a significant removal of zinc but not for iron. The results revealed that for the two treatment, soaking with vinegar for 24h allows a better reduction of phytates with the best bioavailability of iron but low for zinc; soaking in tap water during 24h gives a good bioavailability for iron and a moderate one for zinc. So, we can say that, if intention is to obtain a product rich in iron with less phytates, it is preferable to use 2% acetic acid for 24h which can be adopted in households.

Keywords Cowpea, Phytates, Soaking, Acetic acid, Minerals

1. Introduction

The sub-Saharan Africa population's diet is mainly based on cereals and legumes. Legumes are sources of good quality of protein, carbohydrates, various minerals and vitamins [1]. Having less expensive proteins is an increasing demand across the world and particularly in under-developed countries [2]. Cowpea is one of the main legumes consumed in Senegal which annual production increase from 99924 tons in 2017 to 117784 tons in 2018 [3]. The protein content of cowpea is between 18 and 35 g/100 g [1,4,5]. However, the presence of several antinutritional factors (ANFs) can limit the nutritional values of this legume by reducing the bioavailability of some essential minerals. Phytic acid (phytate; myo-inositol 1,2,3,4,5,6, hexakisphosphate) is one of the ANFs among naturally occurring constituent of plant seeds, roots, tubers, some fruits and vegetables. It acts as a storage form of phosphate [6]. Specifically, phytic acid is known to build complexes with essential dietary minerals such as calcium, zinc, iron and magnesium, making those biologically unavailable for absorption. Phytic acid can also chelate vitamins and potentially contribute to their deficiency and to disease pellagra [7].

In cowpea, phytates content is 559 mg/100g DM [8]. Through previous research, several dipping methods are shown to reduce the phytate content in legume seeds [9,10,11]. Among those methods, soaking, sprouting, fermentation, extrusion cooking and steam pre-cooking are demonstrated [9,11,12]. Soaking cowpea in water during 24 hours lost 8.4% of phytates content [12]. A better reduction of 22.4 and 23.7% on two varieties of cowpea after 24 hours of soaking has been obtained [13]. Unfortunately, these techniques can remove or reduce some recommended components which may be required to enhance nutritional quality [14].

Thus, this study aims to determine optimal conditions which either reduces phytates or preserves nutrients in cowpea.

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2. Material and Methods

2.1. Sampling of Cowpea Seeds

An enough quantity of dry cowpea seeds were purchased from a local food store in Dakar.

2.2. Soaking of Cowpea Seeds

Cowpea samples were processed from the same way for analyzes. They were soaked in order to reduce phytates in seeds. Two types of solutions were used: tap water and acetic acid (vinegar) at 1 and 2% corresponding to 1 and 2° respectively.

Acetic acid is a weak dietary acid which is found in commercial vinegar at 6° ordinary used in households. Treatments were carried out by soaking cowpea in water or acetic acid solutions with the ratio of 1:3 w/v (grain/solution), at room temperature (25°C). Four times soaking were applied: 4, 8, 16 and 24 hours. The pH of tap water was 7.0 and those of the 1 and 2% acetic acid solutions were 4.0 and 3.0 respectively. The samples obtained were named NnT (niébé not treated), NE (niébé soaked in water), NAc1 and NAc2 (niébé soaked in 1% and 2% acetic acid respectively).

2.3. Sample Preparation for Analysis

Beyond soaking essays, cowpea samples were dried at 75°C in an oven during 24 hours. They were finely grounded to a fine powder (particle size of 0.5 mm) using a laboratory mill 3100 (perten instruments) in order to be analysed.

2.4. Analytical Methods

2.4.1. Phytic Acid Quantification

Phytic acid content of cowpea was determined using Latta and Eskin [15] and Vaintraub *et al.* [16] methods with some modifications. 1.2 g of each sample was weighed and introduced into 50 ml tubes. 40 ml of 2.4% concentrated HCl was added to each tube, at room temperature. Tubes were vortexed during 2 hours every 10 min for 15s. After 2 hours, the tubes were centrifuged during 30 min at 3000 rpm. The clear supernatant was used for the phytate quantification. 5 mL of Wade reagent (0.03% solution of FeC13·6H2O containing 0.3% sulfosalicylic acid in water) was added in 15 mL of the supernatant; the mixture is vortexed for 5s. The new supernatant was then transferred in a cell to read absorbance at 500 nm. Distilled water was used as a blank. Phytate content were determined in triplicate.

2.4.2. Zinc and Iron Quantification

Minerals content were determined in triplicate. Zinc and Iron contents were determined by atomic absorption spectrophotometry after mineralization at 550°C for 4 hours. Hydrochloric acid was added to the ash obtained and then evaporated to dryness. The residue was dissolved in Nitric acid and this solution was analyzed by Atomic Absorption Spectrophotometer (AAS) using the technique of flame [17]. The molar ratio acid content was calculated using the formula below:

$$\frac{PA/MW_{PA}}{Min/MWmin}$$

Where: PA = calculated phytate content; MW_{PA} = PA molecular weight (660 Da); Min = mineral content (zinc or iron); MWmin = mineral molecular weight (Zn = 65 Da; Fe = 56 Da).

2.5. Statistical Analysis

Statistical analysis (XLSAT 6.1.9) was accomplished with the ANOVA coupled with the Fisher test at 95% confidence level. Each sample was analyzed in triplicate. The objective is to compare samples contents through each parameter. Microsoft excel 2013 has been used for graphical illustration.

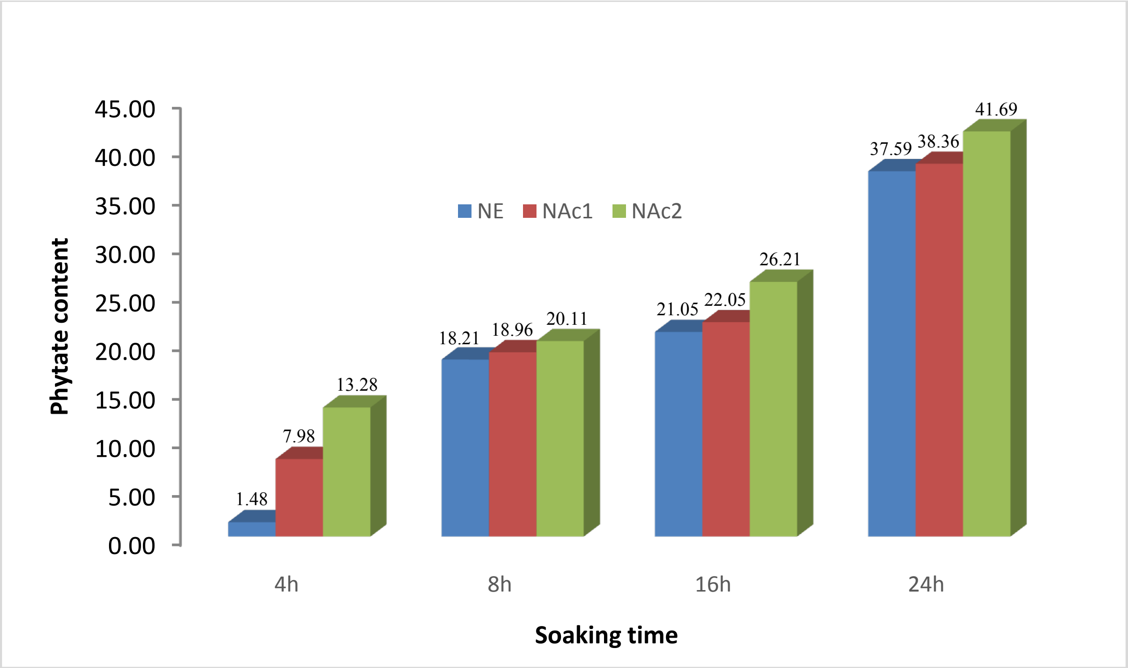
3. Results and Discussion

As shown in Table 1, phytic acid, zinc and iron contents of untreated (not soaked) cowpea are respectively 580.85, 3.22 and 6.48 mg/100g.

Table 1. Phytates, zinc and iron contents of unsoaked and soaked cowpea

Samples	Phytates (mg/100g DM)	Reduction rate (%)	Zinc (mg/100g DM)	Reduction rate (%)	Iron (mg/100g DM)	Reduction rate (%)
nTN	580.85±0.019^{a}	-	3.22a	-	6.48^{d}	-
4NE	572.27±0.147^{a}	1.48	3.01abc	6.52	5.48^{abc}	15.43
8NE	475.06±0.093^{b}	18.21	2.93abc	9.01	5.05^{a}	22.07
16NE	458.59±0.053^{b}	21.05	2.97abc	7.76	5.58^{abc}	13.89
24NE	362.50±0.216^{c}	37.59	3.11ab	3.42	6.37^{d}	1.70
4Nac1	534.51±0.173^{ab}	7.98	2.49de	22.67	5.91^{bcd}	8.80
8Nac1	470.71±0.031^{abc}	18.96	2.65cde	17.70	5.39^{ab}	16.82
16Nac1	452.75±0.006^{bcd}	22.05	2.41def	25.16	5.45^{abc}	15.90
24Nac1	358.04±0.269^{de}	38.36	2.32ef	27.95	9.24^{e}	-42.59
4Nac2	503.73±0.186^{abc}	13.28	2.71bcde	15.84	6.12^{bcd}	5.56
8Nac2	464.02±0.009bcd	20.11	2.75bcd	14.60	6.21^{cd}	4.17
16Nac2	428.61±0.127cde	26.21	2.37def	26.40	6.00^{bcd}	7.41
24Nac2	338.68±0.092e	41.69	2.07f	35.71	9.60^{e}	-48.15

a, b, c, d, e, f, means that values with different letters within a column are different significantly ($P < 0.05$).
Values with a negative sign means that there is an increase.



NE: tap water; NAc1: 1% acetic acid and NAc2: 2 % acetic acid

Figure 1. Rate of phytates reduction during soaking cowpea in variable solutions

For each soaking time, acetic acid solution (vinegar) has a better reduction rate of phytates compared to soaking water.

The content of phytic acid obtained in this study is different with the values reported by Bolade [18] who found more 723, 784, 680 and 980 mg/100g DM in four cultivars of cowpea. However lower value (559 mg/100g DM) is founded by Lestienne [8]. In addition, much lower phytate contents were obtained in five cowpea cultivars which ranged between 117.79 to 254.64 mg/100g [19]. These different levels in phytate contents can be explained by the difference of cultivars used. They can be also attributed to genetic and environmental conditions or the differences methods of quantification.

The results presented in Table 1 show that the phytates content of soaked cowpea grain decreased with the acidity of the soaking solution and the duration of treatment.

The best reduction of phytates content (41.69%) is obtained with acetic acid 2% within 24 hours. Several methods to reduce phytates are already used to reach various levels: distilled water at room temperature by Olika *et al.* [20], tap water by Karkle and Beleia [21] and Ertaş and Türker [22] and sodium bicarbonate solution by Avanza *et al.* [23].

The reduction of phytates is due to their leaching capacity or their hydrolysis by phytases during soaking [24].

Indeed, acidity would facilitate the permeability of membrane walls which became weaked and facilitate the transfer of phytates according to Vijayakumari *et al.* [25]. Acidic conditions can also increase enzymes activities. Phytase's activities were reported to be affected by pH of the soaking solution. In fact, Yoshida *et al.* [26] reported that endogenous phytase have an optimum of action around pH 4–5 while Tomschy *et al.* [27], found a range between 2.5 and 4.5. According to Agte *et al.* [28], phytase reduces the hexa form of phytic acid (IP6, myo-inositol 1,2,3,4,5,6-hexakisphosphate) into lower forms such as IP5, IP4, IP3, IP2, IP1, and myo-inositol. Thus, acidic condition had permitted a reduction of 50% in chickpea [23] and in rice bran [29].

The reduction levels increase with soaking time as reported by Ertaş and Türker [22] and Pereira [30]. For cowpea (*Vigna unguiculata*) it reaches the maximum of 41.69% in 2% acetic acid solution (figure 1). Thus, in practice, reducing antinutritional factors can be done by households by using vinegar solution for 24 hours. However, what about the mineral elements such as iron and zinc?

For minerals, the results (table 1) reveal that soaking in tap water does not reduce significantly neither the zinc nor the iron. The low zinc removal is explained by the strong link with protein in the cells [31]. Acetic acid solutions induced a significant removal of zinc but not for iron. The latest shows a slight increase after 24 hours. This abnormal situation should be due to the reabsorption of this mineral by the grains.

In order to predict the inhibitory effect of phytate on iron and zinc bioavailability in cowpeas varieties, phytate/iron, phytate/zinc molar ratios were calculated (Table 2).

Based on absorption studies in humans, phytate/Zn molar ratios <5, between 5 and 15 and >15 have been associated with high, moderate and low zinc bioavailability, corresponding to approximately 50%, 30% and 15% of total zinc, respectively [32]. While according to Adeyeye [33], a molar ratio phytate/Zn of 10 is the limit for optimal absorption.

Table 2. Molar ratio of phytate/zinc and phytate/iron of cowpea samples

	Molar ratio phytates/zn	Molar ratio phytates/Fe
NnT	17.75	7.47
NE4	18.75	8.71
NE8	15.99	6.68
NE16	15.20	6.85
NE24	11.47	4.74
4NAc1	21.11	8.26
4NAc2	18.30	6.91
8NAc1	17.51	7.69
8NAc2	16.64	6.23
16NAc1	18.52	6.92
16NAc2	17.78	5.95
24NAc1	15.20	3.23
24NAc2	16.14	2.94

These results suggest that the bioavailability of zinc in the most samples may be low because of the higher phytate content. Referring to these values, only NE24 gives a moderate bioavailability of zinc (30%). According to Weaver [31], zinc build the most stable complex with phytate and thus it is the most affected mineral in terms of bioavailability.

Regarding to phytate/iron molar ratio, Lestienne *et al*. [8] considered that diets with phytate/iron molar ratio greater than 10 can be a responsible of iron deficiency. However, in this study all samples have phytate/iron molar ratio less than 10 either for unsoaked or soaked cowpeas. Lestienne *et al*. [8] found a phytate/iron molar ratio of 7 and 8 for unsoaked and soaked cowpea after 24h respectively. The results revealed that for the two treatment, soaking with vinegar for 24h allows a better reduction of phytates with a the best bioavailability of iron but low for zinc; soaking in tap water during 24h gives a good bioavailability for iron and a moderate one for zinc.

4. Conclusions

Soaking is treatment which is commonly used to prepare cowpea in household. The study reveals that soaking promoted significant phytate reduction in acetic acid solution which can be compare to vinegar. This treatment reduced zinc and iron but did not modified seriously their bioavailability. Then the method can be adopted in households.

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