

Original Research Article

Kinetics Approach on the Evolution of the Nutritive Properties, Antinutritional Factors and Free Radical Scavenging Capacity of DPPH during Germination of two Local Legume Varieties (*Phaseolus vulgaris* and *Vigna unguiculata*)

ABSTRACT

Aims: To improve the processes of pre-treatment of legumes for their nutritional valorization.

Study design: Original research.

Place and Duration of Study: This study took place at the Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources, Félix Houphouët-Boigny University between February and July 2022.

Methodology: Red beans and cowpeas purchased on the local market of Adjamé were subjected, after unitary operation of sorting and washing, to a two-factor design of experiment: seed/water ratio and the soaking time, in order to identify the ideal ratio and soaking time to well reduce phytates. Seeds resulting from this pre-treatment were germinated for 72 h and some key nutrient and functional parameters were evaluated.

Results: the ratio 8/9 and soaking time of 18 hours allowed a maximum reduction of 62 and 66.6% of phytate and 72.83 and 67.48% of tanins in cowpea and red beans, respectively. Protein content of these 72 hours germinated pre-treated seeds decreased very slightly and finally remained at high level of 22.02 and 23.13 g/100 g for cowpea and red bean, while reducing sugar levels increased significantly throughout germination to a maximum of 8.19 and 8.13 mg/100 g. Regarding functional and antioxidant properties, a maximum increase in total polyphenols (49.08 and 68.314 mg/100 g) and total flavonoids (13.75 and 39.67 mg/100 g) was observed after 48 h of germination for cowpea and red beans, respectively. Furthermore, this improvement in phenolic content led to a significant improvement in the free radical scavenging capacity of DPPH of 24.50 and 46.38 %. It should also be noted that the germinated red bean showed better nutritional value than the germinated cowpea.

Conclusion: This approach of pre-processing germinated legumes at seed-to-water ratio of 8/9 soaked for 18 hours, providing functional foods with guaranteed nutritional value, appears to be a way to improve local diets.

Keywords: nutrients ; antinutrients ; antioxydants capacity ; germination ; legumes.

1. INTRODUCTION

In developing countries, malnutrition is one of the main problems faced by a large proportion of the population and is a public health problem [1]. Despite the general improvement in food availability, malnutrition persists in various forms, mainly protein-energy malnutrition (PEM). This malnutrition is a major nutritional syndrome affecting over 170 million preschool children and lactating women in developing countries with a prevalence of 50 % in subsaharan Africa [2]. To overcome this problem of malnutrition, dietary approaches have been proposed such as fortification, supplementation, enrichment and diversification [3-5].

Among these approaches, dietary diversification is a strategy to increase the availability, accessibility, consumption and bioefficiency of micronutrient foods. This dietary diversification is based on the use of available, accessible and less expensive food resources such as legumes which are plants belonging to the Leguminosae family that produce seeds in a

pod [6]. Legumes play an important role in agriculture and diet of many developing countries and are a major source of food nutrients for many people. Indeed, legumes are an excellent source of good quality protein with 20-45 % generally rich in the essential amino acid lysine [7] and also have a higher protein content than most plant foods [8]. They are rich in complex carbohydrates and energy [9] with a low glycaemic index (GI) for blood glucose control [10].

In addition, legumes contain a number of bioactive compounds, including phenolic compounds known to positively impact health [11]. Despite this great nutritional potential of legumes, their use seems to be limited due to the many digestive discomforts that follow their consumption. This is strongly related to the low digestibility of proteins, low bioavailability of minerals due to the presence of many anti-nutritional factors [12], which are among others phytates, oligosaccharides and enzyme inhibitors.

It therefore appears necessary to implement an optimised method of substantial reduction of these anti-nutritional factors, less denaturing in order to make the minerals and proteins available. In this respect, numerous techniques such as soaking, cooking, fermentation and germination have been developed to significantly increase the bioavailability of nutrients and thus improve the nutritional value of legumes.

Among these methods, germination is the most used since it is easy to implement. In addition, this method is the most effective in improving the nutritional quality of legumes by reducing anti-nutritional factors and increasing the levels of free amino acids, availability of carbohydrates, dietary fiber and other components of interest [13]. It also increases the functionality of the seeds due to the increase in bioactive compounds [11]. Similarly, it has been reported that the bioavailability of proteins and minerals increases, while that of phytic acid and tannins decreases during legume germination [14].

It should be noted that this technique of reducing anti-nutritional factors is widely used in some countries such as India [15, 16, 12] and China [17, 18], whereas it is hardly used in Côte d'Ivoire where populations consume a lot of these legumes and are regularly confronted with these same digestive problems. In this context, a study was initiated in order to evaluate the best ratio soaking/germination in view to improve the pretreatment processes of legumes for their nutritional valorization through the optimization of the reduction of anti-nutritional factors of two local legumes, red kidney beans (*Phaseolus vulgaris*) and cowpeas (*Vigna unguiculata*), widely consumed in Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1 Materials

Two legumes, red kidney bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) were purchased at the local market in Adjamé (Abidjan, Côte d'Ivoire). The samples were placed in dry labelled plastic bags and transported for analysis to the Biotechnology laboratory of Félix HOUPHOUËT-BOIGNY University in Côte d'Ivoire. Seeds were sorted and cleaned on arrival at the laboratory prior to analysis.

2.2 Methods

2.1.1 Soaking of legumes (cowpea and common bean)

After careful hand-sorting, the legume seeds were soaked in a two-factor, three-stage experimental design, as shown in **Table 1**.

Table 1. 2-factor, 3-level experimental design for the optimisation of legume seed soaking

Soaking experiments	X1 (ratio)	X2 (soaking time h)	Response Y Phytates (mg/100g)
1	1/3	3	Y1
2	2/3	3	Y2
3	1	3	Y3
4	1/3	13,5	Y4
5	2/3	13,5	Y5
6	1	13,5	Y6
7	1/3	24	Y7
8	2/3	24	Y8
9	1	24	Y9

Factor X1: describes the ratio of grain mass to water (minimum 1/3; maximum 1/1)

Factor X2: describes the soaking time (minimum 3 hours; maximum 24 hours)

Response Y: residual phytate content (mg/100 g) in legume seeds

2.1.2 Germination process and flours production

1 kg of legume seeds were soaked for 18 h in distilled water at room temperature (28 °C) to activate the germination process. After soaking, the seeds were drained and spread out on cotton previously placed in baskets and left to germinate in the dark, at room temperature (28-30 °C) and a constant humidity of 80%. During germination, 100 g of germinated seeds were collected every 12 h, washed with distilled water, dried in an oven (Merck, Germany) at 50 °C for 24 h and then crushed using an electric crusher (Moulinex, France). The crushed material was then sieved to obtain flours that were stored at -18°C for further analysis.

2.1.3 Determination of reducing sugars content in legume flours during germination

Reducing sugars content in legumes flours was performed during the different germination time. First, the free water-soluble sugars were extracted using the described method of [19]. The reducing sugars were subsequently quantified by the DNS colorimetric [20] method at 540 nm using a spectrophotometer (PIOWAY, China) and the standard range regression equation established from a glucose stock solution (1 mg/mL).

2.1.4 Determination of Proteins content in legumes flours during the germination time

Kjeldahl method [21] was used for the determination of proteins in samples of sprouted seed flour. After sulphuric digestion in the presence of a catalyst mixture, distillation, absorption of the ammonia in an excess of boric acid solution and titration of nitrogen (N₂) with a standard acid solution, the protein content was estimated using following equation:

$$N_2 (\%) = [(\text{sample titre} - \text{blank titre}) * N \text{ of HCl} * 14 * 100] / (W_s * 1000)$$

$$\text{Protein} (\%) = 6,25 * N_2 (\%)$$

2.1.5 Determination of Total Polyphenols Compounds (TPC) content in legumes flours during the germination time

Total Polyphenols were extracted and determined using Folin-Ciocalteu's reagent [22]. One (1) g of germinated flour was first mixed in 10 mL of methanol 70 % (v/v), then acidified with 0.5% (v/v) HCl 1M and centrifuged at 1000 rpm for 10 min, twice. After centrifugation, supernatants were combined and made up to 100 ml. An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin-Ciocalteu's reagent and neutralized by 1 mL of 20 % (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at room temperature and absorbance was measured at 745 nm using a spectrophotometer (PIOWAY, China) against a blank sample. Total Polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as a standard.

2.1.6 Determination of Total Flavonoids content in legumes flours during the germination time

Total Flavonoids content of germinated flours was evaluated using the method reported by [23]. 0.5 mL of the methanolic extract (1 g of germinated flour mixed in 10 mL of methanol 70 %) was mixed with 0.5 mL of AlCl₃ (10 %, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. The absorbance was measured at 745 nm using a spectrophotometer (PIOWAY, China) after 10 min against a blank sample. Total Flavonoids content was obtained using a calibration curve of quercetin (0,5 mg/mL) as a standard.

2.1.7 Evaluation of the Antioxidant activity of legumes flours during germination

Antioxidant activity assay was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method as described by [24]. To 1 mL of 0.3 mmol/L DPPH solution prepared in ethanol was added 2.5 mL of solution (1 g of flour sample mixed in 10 mL of methanol and filtered through Whatman paper) and was allowed to react for 30 min at room temperature. The absorbance values was measured at 517 nm using a spectrophotometer (Pioway, China) and the average absorbance values were converted to antioxidant activity (%) using the following formula:

$$\text{Antioxidant activity} (\%) = 100 - [(\text{Abs of sample} - \text{Abs of blank}) \times 100 / \text{Abs positive control}]$$

2.1.8 Determination of Phytates content in legumes flours during the germination time

Phytates contents of flours were determined using the Wade's reagent method as described by [25]. To 20 mL of hydrochloric acid (0.65 N) 1 g of germinated seeds flour was added and the mixture was homogenised for 12 h. The mixture was then centrifuged at 12000 rpm for 40 min and 0.5 mL of supernatant was mixed with 3 mL of Wade's reagent. The reaction mixture was stand for 15 min and absorbance was measured at 490 nm using a spectrophotometer (PIOWAY, China) against a blank sample. Phytates content of flours were obtained using a standard calibration curve of sodium phytate (10 mg/mL).

2.1.9 Total Tannins content in legumes flours during the germination time

Total Tannins content of flours were quantified according to method proposed by [26]. For this, vanillin reagent (5 mL) was mixed with 1 mL of the methanolic extract previously obtained and the mixture was allowed to stand for 30 min at ambient temperature. Thereafter, their absorbance was measured at 500 nm using a spectrophotometer (PIOWAY, China) against a blank sample and Total Tannins content was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.

3. Statistical analysis

Data were analysed with SPSS 20.0. The mean and standard deviation of means were calculated using excel and data were analysed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to assess the difference between means that were estimated to be statistically significant with a probability $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Determination of optimal soaking parameters for cowpea and red bean seeds

The experimental design taking into account two factors (seed/water ratio and soaking time) (**Figures 1 and 2**) allowed to determine the seed/water ratio and the soaking time favoring the maximum reduction of the phytate content of legumes.

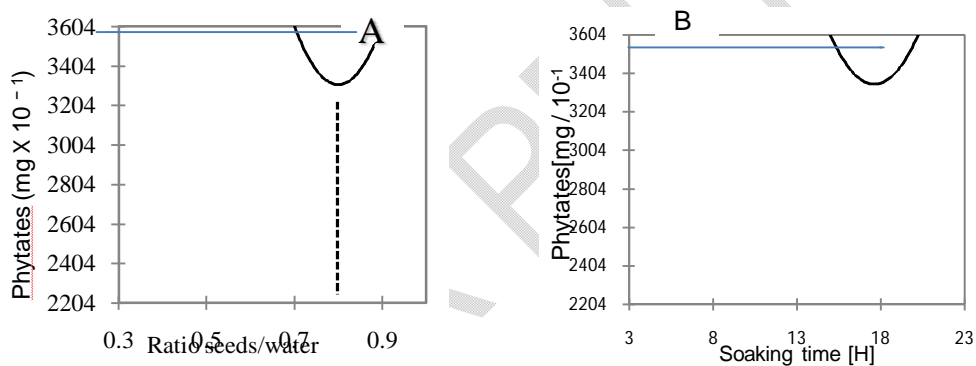


Fig. 1. Optimization curves for grain/water ratio and soaking time of cowpea seeds

A: Optimization of the ratio seeds/water of cowpea seeds; B: Optimization of the soaking time
NB: The initial phytate content of cowpea seeds is 800 mg/100 g

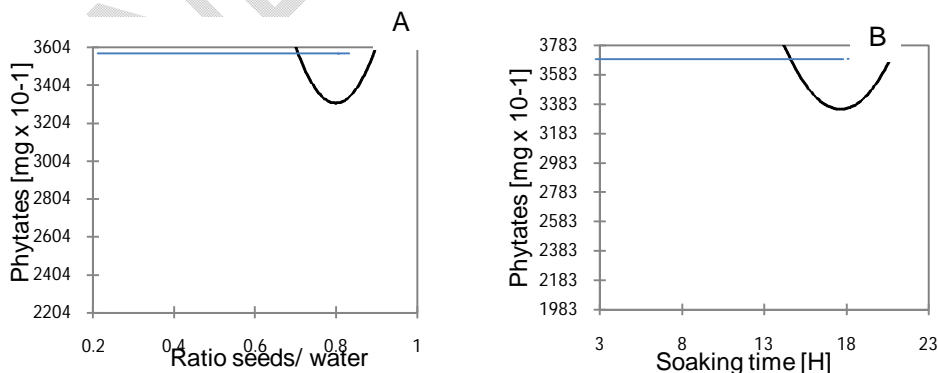


Fig. 2. Optimization curves for grain/water ratio and soaking time of red bean seeds

A: Optimization of the ratio seeds/water of red bean seeds; B: Optimization of the soaking time
NB: The initial phytate content of red bean seeds is 750 mg /100 g

From the analysis of these figures, it can be seen that the ratio (8/9) and the soaking time of 18 hours were the factors that allowed for a maximum reduction rate of 62% of phytates in cowpea (Fig. 1 A and B), while these same parameters allowed for a maximum reduction rate of 66.6% of phytate content for red bean, a reduction of more than half of the initial content (Fig. 2 A and B).

As germination induced several changes in the nutrient content of the plants, it was important to assess its impact on the two legume varieties (*Phaseolus vulgaris* and *Vigna unguiculata*) in the study when the different seeds have been subjected to these previously identified soaking factors. To this end, reducing sugar content of the flours of the two legumes was assessed during the 72 h of germination. Fig. 3 depicted the results obtained. The reducing sugar content of both types of legumes initially low as reported in several studies [27, 28, 29] increased steadily during germination to reach high values after 72 h of germination of 8.13 and 8.19 mg/100 g for red bean and cowpea respectively.

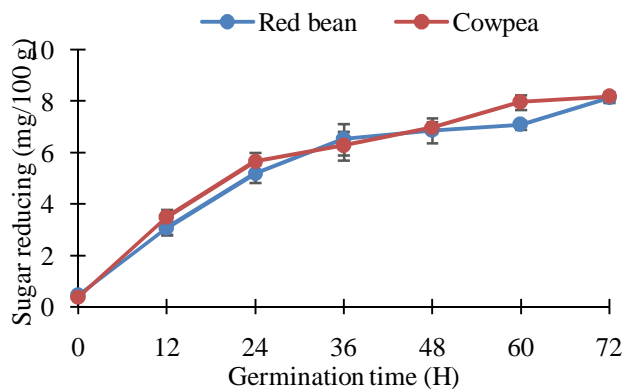


Fig. 3. Effect of germination time on sugars reducing contents of legumes pastes flours

3.2 Monitoring of nutrient and functional parameters during germination of pre-treated seeds

3.2.1 Evaluation of reducing sugar content

Reducing sugar content increased during the 72 h of germination due to the hydrolysis of carbohydrates (sucrose, stachyose, starch) that took place as a result of amylase activation [30]. [31] reported that the generally low levels of glucose and fructose in raw cereals and legumes increase significantly so that when their levels exceed that of sucrose, it activates invertase which hydrolyses sucrose into glucose and fructose. Germination thus induced biochemical changes in carbohydrate macromolecules making sugars available.

3.2.2 Evaluation of total protein content

The protein content of the two studied legumes decreased slightly during the 72 hours of germination but still remains high. Indeed, starting from a value of 23.61 and 22.19 g/100 g (red bean and cowpea flours, respectively), these values have fluctuated to reach levels of 23.13 g/100 g for red bean seeds and 22.02 g/100 g for cowpea seeds at the end of germination. These high protein levels have also been reported in other studies confirming the protein richness of legumes with values between 15 and 30% [32-34].

Table 2. Effect of germination on proteins contents (g/100g) of legumes pastes flours

Temps de fermentation (H)

	0	12	24	36	48	60	72
Red bean	23,61±0,25 ^{aA}	23,55±0,17 ^{abA}	23,35±0,16 ^{bA}	23,39±0,17 ^{bcaA}	23,37±0,17 ^{bcaA}	23,25±0,21 ^{ca}	23,13±0,14 ^{dA}
Cow pea	22,19±0,18 ^{aB}	22,09±0,12 ^{bbB}	22,07±0,16 ^{bcbB}	22,07±0,29 ^{bcbB}	22,05±0,04 ^{cbB}	22,04±0,14 ^{cbB}	22,02±0,22 ^{dbB}

Each value is the mean of triplicate analyses. The same lower case letters in the same line indicate that there is no statistical difference, as do the same upper case letters in the same column ($p < 0.05$ based on Duncan multiple range test)

Therefore, the grain/water ratio of 8/9 and soaking time of 18 h used is ideal because it helped to limit to the maximum the protein losses of the two studied legumes allowing their flours to keep their protein nutritive potential. The observed decrease is in agreement with studies done on *Phaseolus vulgaris* [35] and *Dolichos lablab* [36] germination. However, the losses recorded by these authors were much more pronounced than those in this study, thus reducing the nutritional value of these legumes. Indeed, the availability of protein with a high content in the studied pulses would be beneficial to human health for those who will consume these legumes.

3.2.3 Evaluation of TP, TF content and antioxidant activity

The values obtained for total polyphenol (TP), flavonoid (TF) and antioxidant activity content fluctuated in the opposite way to that of protein until 48 h of germination, after which a decrease was observed until the end of germination for both types of legumes. In addition, red kidney beans showed higher polyphenol, flavonoid and antioxidant activity than cowpea seeds before and during the germination period (Fig. 4).

Total polyphenol contents of red bean and cowpea flours were 45.73 and 26.24 mg/100 g respectively before germination. These values increased steadily throughout germination (Fig. 4A) to reach the maximum content after 48 hours of germination with values of 64.314 and 49.08 mg/100 g, respectively.

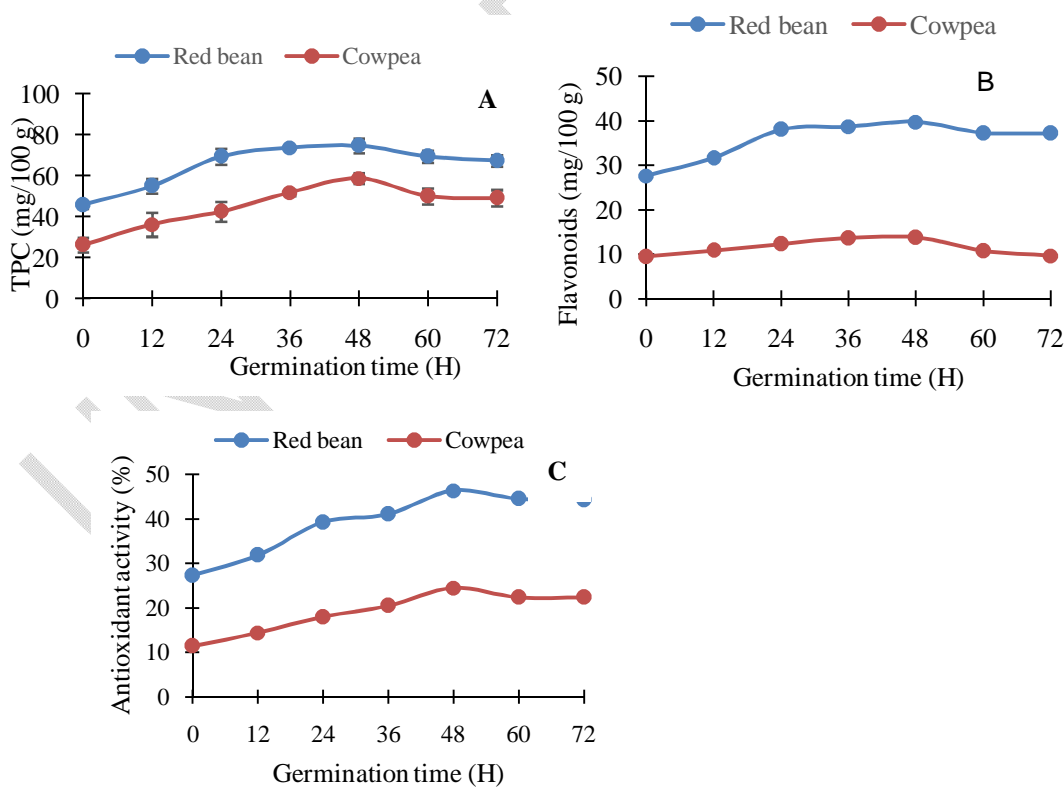


Fig 4. Effect of germination time on total polyphenol (A) and flavonoids (B) and antioxidant activity (C) contents of legumes pastes flours

Then, they decrease slightly thereafter to remain stable until the end of germination. As for flavonoid content of the flours of the two studied legumes, a similar trend was observed during the 72 h of germination with values initially of 27.60 and 9.47 mg/100 g, respectively, which increased to maximum values of 37.11 and 13.75 mg/100 g after 48 h for red bean and cowpea flours, respectively (Fig. 4B). This important increase of these functional parameters in the studied legume flours underlined the necessity to adopt soaking at the indicated ratio of seeds and water and time to improve the nutritional and health contribution of our foods.

The positive correlation between polyphenol and flavonoid content and antioxidant activity is remarkable (Fig. 4C). Indeed, as well as total polyphenol and flavonoid contents, the antioxidant activity obtained for the germinated seed flours of both types of legumes initially of 27.35 and 11.44% before germination increased to maximum values of 44.27 and 22.42% after 48 h of germination.

Several studies have reported enhanced levels of total polyphenols, flavonoids and antioxidant activity during legume germination. Studies by [37] reported an increase in total polyphenol content of *Cajanus cajan* L. seeds during 5 days of germination from 22.8 to 115 µg/100 g. Similarly, the work carried out by [38] on changes in the nutritional composition and antioxidant capacity of chia seeds (*Salvia hispanica* L.) during the germination process reported an increase in the content of total polyphenols, total flavonoids and antioxidant activity during 4 days of germination. These contents increased from 97.7 to 293.6 mg/100 g for total polyphenols, from 35.8 to 106.0 mg/100 g for total flavonoids and finally for antioxidant activity from 41.1 to 82.9 µmol/g [38]. The significant increase in total phenolic content (polyphenolic compounds and flavonoids) observed during germination could be due to the activation of enzymatic syntheses that takes place during germination following the lifting of seed dormancy [39]. Thus, the excellent correlation between the DPPH method and flavonoids and polyphenol, underlines the concordance between these phenolic compounds and antioxidant activity in legumes. This further indicated that these phenolic compounds would be the main contributors to the antioxidant properties of these plants. Anyway, germination induced a structural breakdown of cell walls, which may result in the increase of the bound phenolic content [40]. During the germination process of plants, synthesis of phenolic compounds plays an important role in structural growth and in protecting the plants from abiotic and biotic stresses [41]. The consumption of polyphenol-rich foods has been associated with body wellness in that it has been associated with a reduced risk of a number of chronic diseases, including cancer, cardiovascular disease (CVD), and neurodegenerative disorders [42]. These flours could therefore contribute to the good health of sub-Saharan African populations.

3.2.4 Evaluation of anti-nutritional factors

Legumes naturally contain anti-nutritional factors, including phytic acid and tanins, which limit the bioavailability of minerals and proteins [43] causing many of the digestive discomforts that occur when consuming them. The results presented in **Table 3** depicted the effect of germination on content of two main anti-nutritional factors including tanin and phytate on legume flours during the 72 h of germination. Analysis of this table revealed that germination significantly reduced their content in phytate and tanin.

Table 3. Tanins and phytates composition (mg/100 g) of red beans and cowpea seeds germinated for different time period

Germination time (h)	Tanins (mg/100 g)		Phytates (mg/100 g)	
	Red beans	Cowpea	Red beans	Cowpea
0	15.24 ± 0.17 ^a	15.13 ± 0.13 ^a	331.00 ± 0.96 ^a	335.56 ± 3.15 ^a
12	14.44 ± 0.02 ^b	14.06 ± 0.05 ^b	253.05 ± 1.64 ^b	319.80 ± 3.19 ^b
24	13.92 ± 0.10 ^c	13.53 ± 0.10 ^c	221.66 ± 1.17 ^c	281.92 ± 2.98 ^c
36	12.72 ± 0.01 ^d	11.79 ± 0.11 ^d	222.90 ± 0.57 ^d	264.57 ± 1.08 ^d
48	11.84 ± 0.06 ^e	10.58 ± 0.17 ^e	211.03 ± 0.63 ^e	223.47 ± 2.07 ^e
60	11.10 ± 0.01 ^f	10.22 ± 0.01 ^f	204.34 ± 0.59 ^f	223.41 ± 1.52 ^f
72	11.10 ± 0.09 ^f	10.21 ± 0.03 ^f	205.10 ± 0.54 ^f	223.39 ± 2.15 ^f

Data are means of three determinations (n = 3) ± SD. Means with different superscripts in each column indicate significant differences at p ≤ 0.05 based on Duncan multiple range test.

Regarding phytates, from an initial value of 331 and 335.56 mg/100 g for red beans and cowpea respectively, these values dropped to 205.10 mg/100 g and 223.39 mg/100 g, respectively, at the end of germination. As concern tanins, the obtained values at the end of germination were 11.10 and 10.21 mg/100 g respectively for Red beans and cowpeas.

Overall, we noted that the longer the germination time, the more anti-nutritional factors were eliminated (72 h germination < 48 h germination < 24 h germination < raw beans). Similar results have been found in many studies [44, 39]. The decrease in tannin levels in legumes during germination can be attributed to the increase in polyphenol oxidase activity and other catabolic enzymes that lead to hydrolysis of various components [45]. Phytate losses can also be attributed to the activation of endogenous phytase [39]. Thus, the soaking of the different studied legumes at the 8/9 ratio (seeds/water) for a time of 18 h allowed an optimal reduction of more than half (62% and 66.6% (cowpea and red beans) and 72.8% and 67.55% (cowpea and red beans) of the initial contents of phytates and tannins should be recommended to these different parameters to sustainably improve the quality of food prepared from these legumes. This as this practice could help make minerals and nutrients more bioavailable and thus increase the nutritional value of foods (Oghbaei & Prakash, 2016) made from these legumes.

4. CONCLUSION

The optimum soaking parameters identified in this study, i.e. a grain/water ratio of 8/9 for a soaking time of 18 h, allowed a maximum reduction of more than 60% of phytates and tannins in cowpea and red bean flours produced after 72 h of germination. This significant reduction of these anti-nutritional factors was coupled with an increase in the content of certain key nutritional compounds such as total polyphenols, total flavonoids combined with an increase in the free radical scavenging capacity of DPPH until 48 h of germination and an increase in the total sugar content tout au long des 72 h de germination. On the other hand, a slight decrease in protein content was observed in both flour trials, although the residual protein content was still high (23.13 and 22.02 %) after 72 h of germination. Thus, the 72 h germination process coupled with the soaking of the 8/9 grain/water ratio for 18 h appears to be effective in improving the nutritional quality and palatability of red beans and cowpeas.

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