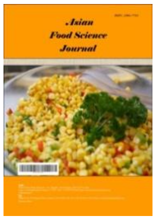

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Effect of Germination on the Physicochemical and Antinutritionnal Parameters of White Beans (*Phaseolus vulgaris L.*) Seeds Cultivated in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of the present study was to evaluate some physicochemical and some antinutritional factors characteristics of sprouted seeds of white bean (*Phaseolus vulgaris* L.) with a view to their valorization in the Ivorian diet. The contents of dry matter, ash, reducing sugars, total sugars, fat, increased significantly under the effect of the germination of bean seeds. In addition, the mineral contents (Calcium, Magnesium, Iron, Sodium, Potassium) of sprouted bean samples are

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statistically different from those of ungerminated beans. Then, it was found that germination resulted in a significant increase in the contents of vitamins (A, B1, C, D and E). However, a significant decrease in the anti-nutritive compounds such alkaloid, oxalates, phytates and tannins was observed in the sprouted bean seeds. The mean values range from 45.00 ± 3.54 to 16.25 ± 1.28; from 68.29 ± 0.71 to 21.32 ± 0.18; from 81.72 ± 2.48 to 38.14 ± 1.76 and from 51.58 ± 0.26 to 19.35 ± 0.53 per cent respectively. Thus, germination is an effective processing method for increasing vitamins and mineral bioavailability, and for reducing significantly anti-nutritve compounds after the bean seeds germinate.

Keywords: Germination; Phaseolus vulgaris L.; physicochemical; antinutritional parameters.

1. INTRODUCTION

Legumes are the third largest angiosperm family belonging to Fabaceae [1]. They are important components of a healthy diet and feature prominently in traditional diets around the world [2,3].

The white bean (*Phaseolus vulgaris* L.) with an estimated production of 29.27 million tonnes is the most produced and consumed legume in the world [4] then occupies an important place in human nutrition in the regions of the Africa and particularly in Côte d'Ivoire [5-7]. Indeed, the white bean is an essential plant for a good nutritional balance and constitutes a good base of the protein intake of plant origin necessary for each individual [8]. In addition, the white bean is a very interesting plant from the nutritional point of view because of its richness as well in proteins as in certain minerals, in carbohydrates and in vitamins. However, the presence of anti-nutritional compounds can affect the digestibility of protein as well as other nutrients [9,10]. In addition, some anti-nutritional factors such as α-galactoside, the fermentation of which in the colon is largely responsible for gas [11-13]. This remark is one of the main reasons why people are turning away from the consumption of legumes and especially beans. However, all of this information on the nutritional potential of beans only relates to grains in the raw state. This suggests that there is still an information gap to be filled, particularly in terms of the impact of germination technologies on the nutritional value of bean kernels [14]. It is with this in mind that this article focuses on some physicochemical and antinutritional factors of sprouted seeds of white bean (*Phaseolus vulgaris* L.) cultivated in Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Raw Material

The raw material (Fig. 1), the white bean seeds (*Phaseolus vulgaris* L.) were purchased on local market of Daloa (Côte d'Ivoire).

2.2 Germination Method

Three hundred grams of the sorted bean sample, disinfected with 1% (v/v) sodium hypochlorite for 10 minutes, is washed thoroughly in tap water and soaked for 24 hours in 500 milliliters of water contained in a 2 liter plastic bucket. They are then spread out on a 100 % cotton cloth and placed in a plastic pot in a room with humidity and temperature of around 85 % and 28 °C respectively (Fig. 2). Every day, the germinating seeds are watered only once [15]. The seeds germinated for three (3) days and were prepared for the flours and assay for enzymatic activities (Fig. 2).

2.3 Flour Production

The bean seed samples were oven dried for 48 hours at 45 °C. They were then ground in a MOULINEX brand mixer to obtain a flour according Aguemon et al method [16]. This flour was stored at room temperature in previously dried jars for possible analysis.

2.4 Proximate Analysis

Total ash was determined by incinerating in a furnace at 550 ºC for 24 hours [17]. The lipid content of each bean flour sample (non-germinated and sprouted) was determined by hexane extraction with SOXHLET for 6 hours at 69-70 °C [17]. The dry matter content was determined by the AOAC method [17], the principle of which is based on dehydration by drying in an oven at 105 °C (± 2 °C) for 24 hours of the samples up to obtaining a constant mass. Crude protein content was subsequently calculated from nitrogen (Nx6.25) obtained using the Kjeldahl method by AOAC [17]. The carbohydrate content was determined by deference that is by deducting the mean values of other parameters that were determined from 100. Therefore % carbohydrate = 100- (% moisture + % crude protein + % crude fat + % ash). The quantification of reducing sugars was carried out according to the method of Bernfeld [18] using 3.5 dinitrosalycil acids while the determination of total soluble sugars was carried out according to the method described by Dubois et al. [19]. The starch level is determined by the difference between the percentages of total carbohydrates and total sugars. The pH was determined according to the AFNOR method [20].

2.5 Mineral Analysis

The method described by AOAC [17] was used for mineral analysis. Flours were digested with a mixture of concentrated nitric acid (14.44 mol/L), sulfuric acid (18.01 mol/L) and perchloric acid (11.80 mol/L) and analysed using an atomic absorption spectrophotometer.

2.6 Vitamins Dosage

Analytical technique used for determination of water-soluble vitamins thiamin (B1), ribbyoflavin, niacin and folate folate content is HPLC-method [21]. Vitamin C (ascorbic acid) content was determined by the method of Pongracz et al. [22] and Barros et al. [23].Vitamin D, vitamin E (α-tocopherol, γ-tocopherol), vitamin K (phylloquinone) and carotenoids (provitamin A = β-carotene,) were extracted from 500 mg of white beans flour using the following method: 2 mL of distilled water were added to sample. The internal standard (retinyl acetate) was added to the sample in 2 mL of ethanol. The mixture was extracted twice with 8 mL of hexane. After centrifugation (500× g, 10 min at 4 °C), 2 mL of distilled water and methanol–dichloromethane (65/35, v/v). A final volume of 150 µL for samples was used for HPLC analysis. The fat-soluble vitamins and carotenoids were separated as previously described [24,25]. All molecules were identified by retention time compared with pure standards.

2.7 Antinutrients Analysis

Alkaloid content was estimated by the filtration method of Harbone [26]. Oxalate content was determined by the modified method of Ukpabi and Ejidoh [27]. Tannin content was estimated by the colometric method described by Makkar et al. [28]. Phytate content was determined by the spectrophotometric method described by Mohamed et al. [29]. Lignin content was estimated by Saura-Calixto et al. [30] method. Each of the samples from Dioscorea esculenta flour was analyzed in triplicate for their physico-chemical and antinutrients properties.

2.8 Statistical Analysis

All measurements were performed in triplicate. Statistical analyzes of the data were performed using STATISTICA 7 software (Statsoft Inc, Tulsa-USA Headquarters). Comparisons between dependent variables were determined using analysis of variance (one-way ANOVA) and Duncan's test according to the general linear model. The difference between two variables is significant if $p \leq 0.05$.

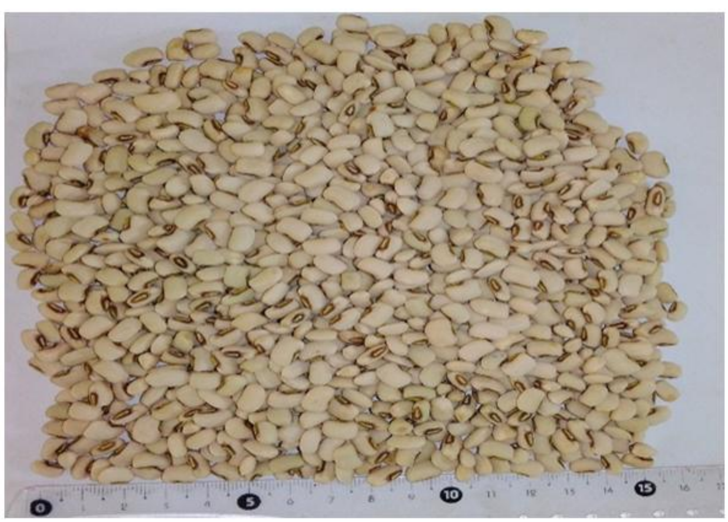

Fig. 1. White bean (*Phaseolus vulgaris* L.)

Fig. 2. Sprouted white bean (*Phaseolus vulgaris* L.) seeds

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The physicochemical characteristics of bean flours are presented in Table 1. The contents of proteins, reducing sugars, total sugars, total carbohydrates, dry matter, the ash and starch levels and then the pH increase significantly ($p \leq 0.05$) with the process of bean seed germination.

The protein content ranges from 21.20 ± 0.20 to 26.80 ± 0.60 % respectively for ungerminated bean and sprouted bean (Table 1). This same observation was observed by Kassegn et al. [31]. Indeed, these authors found that after 72 hours of germination, the protein content increased by 3 to 4 % in the bean seed. This increase in protein content could be attributed to the synthesis of enzymatic proteins (proteases) by seed germination [32-34]. According to Nonogaki et al. [35] protein synthesis occurred during imbibitions. Therefore, the increase in protein content is thought to be due to the synthesis of amino acids during germination [36-38]. Thus, germinated seeds contain more nitrogen than non-germinated seeds [39]. Furthermore, Faltermaier et al. [40] reported that legumes have low endogenous endopeptidase activity, which however increases considerably after one to three days of germination. In addition, protein solubility and digestibility are higher during the first days of germination [41]. The protein contents obtained are much higher than that of okra (2.10g / 100g DM) found by [42] [43]. Thus sprouted bean seeds would then be a good source of protein.

Statistical analysis did not reveal a significant difference in the fat content of bean flours (non-sprouted and sprouted) (Table 1). However, studies have found that germination reduces fat content [37] due to hydrolysis and the use of fat as an energy source for biochemical reactions during germination [44] [38]. The values obtained are lower than those of *Beilschmiedia mannii* (spicy cerdres) which has a value of 2.04g/100g DM [45]. Therefore, the sprouted seeds could be used in the diet of people who are not advised to eat fatty foods.

As for total carbohydrates, there is a significant difference between the values of ungerminated bean (64.66 ± 1.08%) and sprouted bean (60.15 ± 0.52%) (Table1). The observed decrease in carbohydrate content is thought to be due to the enzymatic breakdown of carbohydrates into simple sugars through activation of endogenous enzymes such as α-amylase thereby improving digestibility [46] as a result of degradation of starch to provide energy for the seed development [38] [47]. Indeed, germination facilitates the breakdown of starch to provide energy for seed development [46]. The total carbohydrate contents of the present bean seeds are similar to those reported by Barros and Prudencio [48] whose work is focused on certain bean varieties. Thus, the consumption of bean sprouts should be encouraged for their high carbohydrate content.

The starch rate indicated in Table 1 is high at the level of sprouted beans (58.86 ± 1.21 %). According to these same authors, germination increases the proportion of slowly digestible starch. However, El-Adawy et al. [49] reported in their work on legumes that starch proportions decrease after three days of germination.

The proportion of ash (Table 1) increased considerably from 2.74 ± 0.25 % (non-sprouted bean) to 4.26 ± 0.25 % (sprouted bean). This same observation was observed by El-Adawy et al. [50] then Shah et al. [33]. Indeed, according to Mostafa [51], the increase in ash content is only apparent and attributable to the disappearance of starch. The proportion of ash obtained for sprouted beans is similar to those mentioned (4.16-4.90 %) by Mostafa [51], whose work concerned legumes. However, the present proportion of ash is higher than that reported (2.75 %) by Kassegn et al. [31] on bean seed after 72 hours of germination. This would assume that the flour from the sprouted bean seeds is a good source of important minerals.

Sprouted bean flour has high levels of total sugars and reducing sugars compared to that of ungerminated bean (Table 1). It should be remembered here that according to Samia El-Safy et al. [49] the increase in total and reducing sugars contents after germination would come mainly from starch hydrolyzed by amylases. The results obtained corroborate those of Tian et al. [52]. This is because sugars are the body's main source of energy. They allow the proper functioning of muscles and eyes. The values obtained corroborate those reported by Samia El-Safy et al. [49] Tian et al. [39] whose work focused on legumes. Thus, these sugars contents suggested the consumption of sprouted bean seeds.

3.2 Mineral Composition

The germination of bean seeds considerably increased the contents of calcium (65.00 ± 2.00 to 94.00 ± 2.00 %), magnesium (95.00 ± 3.00 to 111.00 ± 0.20 %), potassium (2.30 ± 0.30 to 4.50 ± 0.20 %), iron (0.70 ± 0.02 to 1.93 ± 0.03 %) and sodium (0.60 ± 0.10 to 0.80 ± 0.12 %) (Table 2). According to Luo et al. [53], this remark would be attributed to the increase in phytase activity during germination which helps to reduce phytic acids that bind to minerals, subsequently leading to increased availability of minerals. This is because legumes contain an endogenous phytase enzyme which is activated by germination to destroy phytate [53] [38]. In addition, it should be remembered that the significant increase in the bioaccessibility of minerals shows the decrease in the content of antinutritional compounds [49]. The present results corroborate those obtained by El-Adawy et al. [50], Laxmi et al. [34] and Nkhata et al. [38] whose work focused on legumes. Consequently, the consumption of the present germinated bean seeds would then be recommended for the prevention of certain diseases [54] [55].

3.3 Vitamin Composition

The vitamin contents in bean seeds increased after the germination process (Table 3). This same observation was observed by Kim et al. [52], Ahmed et al. [56] as well as Lemmens et al. [57] for various vitamins present in legumes. According to Mostafa [51], certain vitamins such as vitamin A and vitamin E are produced during the germination process of legumes. In addition, Laxmi et al. [34] found an increase in vitamin C content in sprouted samples of legumes.

3.4 Antinutrient (Alkaloid, Oxalate, Phytate and Tannin) Contents

The nutritional importance of a given food depends on the nutrients and anti-nutritional constituents of the food [58]. Thus, the anti-nutrient composition of flour from ungerminated and germinated flours is shown in Table 4.

Table 1. Physicochemical characteristics of bean flours (non-germinated and sprouted)

Composition (%) DM	Ungerminated Beans	Germinated Beans
Dry matter	89.20 ± 0.50^{a}	92.07 ± 0.20^{b}
Ash	2.74 ± 0.25^{a}	4.26 ± 0.25^{b}
Total sugar	6.50 ± 0.40^{a}	7.86±0.30^{b}
Carbohydrates	64.66 ± 1.08^{b}	60.15 ± 0.52^{a}
Crude Fat	0.60 ± 0.20^{a}	0.86 ± 0.30^{a}
Starch	57.71 ± 0.26^{a}	58.86 ± 1.21^{b}
Reducing sugar	4.30 ± 0.30^{a}	6.50 ± 0.40^{b}
Crude Protein	21.20 ± 0.20^{a}	26.80 ± 0.60^{b}
pH	6.46 ± 0.02^{b}	5.63 ± 0.01^{a}

Table 2. Some minerals in ungerminated and germinated beans

Parameters	Values (% DW)		Percentage increase (%)
	Flour from ungerminated beans	Flour from germinated beans	
Calcium (Ca)	65.00 ± 2.00[a]	94.00 ± 2.00[b]	144.62
Magnesium (Mg)	95.00 ± 3.00[a]	111.00 ± 0.20[b]	116.84
Iron (Fe)	0.70 ± 0.02 [a]	1.93 ± 0.03 [b]	275.71
Potassium (K)	2.30 ± 0.30[a]	4.50 ± 0.20[b]	195.65
Sodium (Na)	0.60 ± 0.10[a]	0.80 ± 0.12[b]	133.33

Table 3. Vitamins in non-sprouted and sprouted beans

Parameters	Values (% DW)		Percentage increase
	Flour from ungerminated beans	Flour from germinated beans	
Vitamin A	3.00 ± 0.25[a]	6.00 ± 0.02[b]	200.00
Vitamin B1	0.03 ± 0.01[a]	0.08 ± 0.02[b]	266.67
Vitamin C	7.00 ± 1.00[a]	9.60 ± 0.60[b]	137.14
Vitamin D	6.50 ± 0.40[a]	7.86 ± 0.30[b]	120.92
Vitamin E	2.1 ± 0.20[a]	3.40 ± 0.40[b]	161.90

Table 4. Antinutritional factors from non-sprouted and sprouted beans flour

Parameters		Values (% DW)	
		Flour from ungerminated Beans	Flour from germinated Beans
Antinutritional Contents	Alkaloid	45.00 ± 3.54[b]	16.25 ± 1.28[a]
	Oxalate	68.29 ± 0.71[b]	21.32 ± 0.18[a]
	Phytate	81.72 ± 2.48[b]	38.14 ± 1.76[a]
	Tannin	51.58 ± 0.26[b]	19.35 ± 0.53[a]

The alkaloid, tannin, oxalate and phytate contents decreased and ranged from 45.00 ± 3.54 % dw to 16.25 ± 1.28 % dw, from 68.29 ± 0.71 % to 21.32 ± 0.18 %, from 81.72 ± 2.48 % to 38.14 ± 1.76 %, and from 51.58 ± 0.26 % to 19.35 ± 0.53 % for the flour from raw and germinated respectively (Table 4). Thus, the highest values were obtained with raw white beans and the lowest values with the germinated white beans. Indeed, germination reduced significantly ($p \leq 0.05$) all the antinutrients factors after three days. The availability of alkaloids in the white beans indicates that beans cannot be eaten raw. Most alkaloids are known for their pharmacological effects rather than their toxicity. However, when alkaloids occur in high levels in foods, they cause gastro-intestinal upset and neurological disorders [57].

The total oxalate contents of flour from raw and germinated beans decreased significantly ($p \leq 0.05$) during the germination. Besides, the higher percentage of oxalate reduction in the value of the oxalate contents of flour from beans during germination may also be due to its solubility in germination water. Germination may cause considerable skin rupture and facilitate the leakage of soluble oxalate. This may be the possible reason to observe high reduction in oxalate level up after germination [59]. Otherwise, the reduced oxalate content on germinated beans could have positive impact on the health of consumers. The reduction of oxalate levels after germination is expected to enhance the bioavailability of essential dietary minerals of the tubers and reduce the risk of kidney stones occurring among consumers [60] [61].

Concerning the phytate content, there was a significant difference ($p \leq 0.05$) among the samples during germination. Indeed, the phytate content decreased significantly ($p \leq 0.05$) after three days germination. Furthermore, the knowledge of the phytate level in foods is necessary because high concentration can cause adverse effects on the digestibility [62]. This is important because high phytate content is of significance as it lowers the availability of many essential minerals. Thus, according to

Schlemmer et al. [63], most phytic acid is chelated by (divalent) cations, such as those of iron (Fe), zinc (Zn), calcium (Ca), manganese (Mn), magnesium (Mg), and copper (Cu), to result in phytates. Phytate could be substantially reduced or eliminated by soaking, cooking and germination [64]. Thus, during germination of cereal and legumes seeds, phytases are activated, and secreted to make phosphate, mineral elements, and myoinositol available for plant growth and development [65]. In general, higher endogenous phytase activity levels in a cereal and legume result in more extensive phytate hydrolysis. Therefore, relatively long sprouting times (3 to 5 days) are needed to lower the phytate concentration by more than 30 % [66] [67].

According to Jambunathan and Singh [68], tannins are known to inhibit the activities of digestive enzymes and hence the presence of even a low level of tannin is not desirable from nutritional point of view. Thus tannins affect the nutritive value of food products by forming insoluble complexes with proteins thereby decreasing the digestibility of proteins [69] [70]. Tannins may decrease protein quality by decreasing digestibility and palatability, damaging the intestinal tract, and enhancing carcinogenesis [69]. They also bind iron, making it unavailable [71]. Thus, a decrease in tannin content of 8 % to 60 % has been noted when sprouting sorghum [72]. This has been attributed to leaching of tannin into the steeping medium, to the activity of polyphenol oxidases, and to polymerization of tannins, the latter resulting in loss of solubility [72]. Owing to the reduction in the tannin and phytic acid content in malted cereals, minerals are made more bioavailable, thereby increasing the nutritional value of the food [73] [47].

4. CONCLUSION

The present study was performed to demonstrate the effect of germination on the physicochemical and enzymatic properties of bean seeds. The results of this study show that germination, as a biotechnological technique, induced changes in biochemical compositions, enzymatic activities, minerals, vitamins, functional properties and digestibility on bean seeds. Unlike ungerminated seeds, sprouted bean seeds are more digestible with relatively high levels of total carbohydrates, dry matter, magnesium, calcium, potassium, vitamin (A, B1, and E). However, the flour obtained from the bean sprouts should be supplemented with local fruits and vegetables, which are rich in other vitamins and minerals. Evidence is overwhelming indicating that germinated foods are superior in nutrients compared to their ungerminated counterparts due to activation of endogenous enzymes that degrade antinutritional factors. Therefore, optimum germination conditions should be determined for each cereal and legume in order to optimize the health and nutrition benefit of that process.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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