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Pathways for Reducing Anti-Nutritional Factors: Prospects for Vigna unguiculata

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Abstract

Cereals, pulses and legumes are the most consumed foods in Sub-Saharan Africa. Cowpea, *Vigna unguiculata* (L.) walp is one known of these legumes as a good source of proteins, some minerals, and fibers. However, the presence of anti-nutritional factors (ANFs) including phytates, alpha - galactosides such as raffinose and stachyose, and tannins reduce significantly the micronutrients and also create some problems of acceptability in using cowpea and other legumes. This review article rep the current knowledges on key aspects of technologies used to reduce ANFs. Some of them proposed one either pathway or combining many techniques such as soaking, sprouting, fermentation, extrusion cooking and steam pre-cooking in order to reduce these ANFs. In fact, steam pre-cooking seems to be the most promising among established processes because of its potential to reduce ANFs up to 96%. Nevertheless, soaking can reduce ANFs up to 45% with higher reductions by adding sodium bicarbonate. Sprouting however can record more significant reductions of 33 to 72% of tannins, 96% of phytates, 92% of stachyose and 67% of raffinose. With regard to fermentation, a reduction rate in ANFs exceeding 50% was found. The extrusion technic reduces up to 55.83% at 140 ° C and 20% humidity while the steam pre - cooking resulted reductions of up to 52.60% at 110 ° C for 25 minutes. In this regard, it is critical to investigate mechanisms that would improve these reductions and furthermore, investigate all strategies used to increase bioavailability of nutrients under specific technological condition.

Keywords: Cowpea; anti nutritional factors; soaking; sprouting; fermentation; extrusions; steam pre – cooking

Introduction

Cereals and legumes are the most important consumed food based products in sub-Saharan Africa. Cowpea, *Vigna unguiculata* (L.) walp, is one well known legumes as an important source of protein for millions of people living in semi-arid countries [1]. It is a good legume for drought tolerance. Globally, West Africa has the largest production and consumption of cowpea including Senegal, where cowpea is the most widely grown legume seed after groundnuts [1, 2]. Thus, the annual production of cowpea is 99 924 tons and 117 784 tons respectively in 2017 and 2018 [2, 3]. Nutritionally, mature cowpea seeds contain proteins, starch, vitamins B such as folic acid, which is important in preventing malformation in newborns [4]. In addition, cowpea proteins are highly digestible with important biological values [5]. Furthermore, cowpea seed contains 88.7g / 100g in Dry Matter (DM). This dry matter is composed by 1.2g / 100g in lipids, 24.3g / 100g in proteins, 31.0g / 100g in total fibers and 3.2g / 100g in ash, 50-67% starch [4, 6]. Cowpea seed has also good amounts of iron and zinc respectively 8 mg and 5 mg / 100 mg DM [7]. In addition, have shown that the consumption of one kilogram of cowpea seeds cover the daily energy allowance for an adult man, which is 3050 kcal [8].

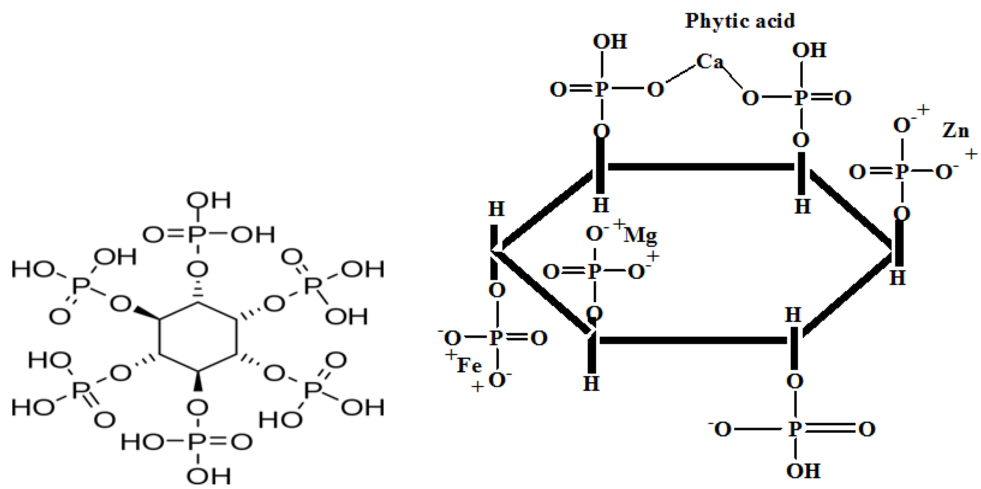
However, the presence of ANFs may negatively affect the acceptability of cowpea based food products, their proteins bioavailability, and minerals as well as in other legumes. According to, ANFs are found in almost all foods. However, several technological processes are used in order to reduce these ANFs and to provide consumers nutritional rich food products [9]. Moreover, these technological processes need to be optimized for more effectiveness. The purpose of this review paper covers the current and modern practices on key aspects of these technologies in reducing or eliminating ANFs in legumes. This state of art cross over above technologies could be also applied to *Vigna unguiculata*.

Structure and Nutritional Effects of Some Major Antinutrients Factors (Anfs) in Legumes

Plants have substances that can negatively impact digestion, nutrient uptake, and metabolism [10, 11]. These substances are mostly called antinutritional factors [12]. They reduce the absorption of micronutrients from plant based food products [13]. Thus, different ANFs are found in legume seeds and particularly in cowpea include phytates, tannins, andalpha-galactosides including raffinose, stachyose and verbascose [14, 15].

Phytates

Phytates are salts of phytic acids also called myo-inositolhexa-phosphoric acid. With formula of C6H18O24P6, this acid has an esterified inositol radical with six phosphate radicals Figure 1a. The amount of phytates in different legume samples differs from varieties ranging from 8.10 to 16.36 mg / g DM [6, 16]. In cowpea seeds the phytate content can reach 836 mg/ 100g and even further to 1230 mg / g DM [17, 18]. The location of phytates in plants varies with the types. In legume seeds, they are stored in cotyledons [19]. Thus, in most seeds, phytates constitute the primary phosphate reserve having 60-90% of total phosphorus [20]. Phytates greatly influence functional and nutritional properties of food products by binding to minerals such as calcium, magnesium, copper, iron and zinc Figure 1b [21]. Thus will decrease their bioavailability. Phytates have a strong binding capacity and can therefore form complexes with proteins and multivalent cations that affect digestion. Indeed, studies have shown that most phytate metal complexes are insoluble at physiological pH rendering minerals not bioavailable [22]. However, all minerals haven't the same affinity for phytates and so there may be competition if the phytate / mineral balance is disrupted. Thus, the stability of phytates and their affinity for cations vary as follows: Fe < Ca < Mn < Co < Cu < Zn [23]. The trace elements of nutritional interest are more strongly fixed by the phytic acid than the alkaline-earth macronutrients. However, because phytic acid is less digested by monogastric animals and by humans, and it interacts with amino acids and also chelates minerals such as Fe^{3+}, Zn^{2+}, and Ca^{2+}, a high amount of phytic acid decreases the absorption of nutrients and their bioavailability. There are reported by that phytate molar ratio over zinc (Phy / Zn) greater than 10 or 15 progressively inhibited zinc uptake in rats fed diets of egg albumen supplemented with phytates or zinc [24]. They also reported that radioactive iron absorption in rats decreased significantly when phytate-to-iron molar ratios (Phy / Fe) were greater than 10-14 in wheat flour diets containing between 0.19 and 1. 85 g phytates/100 g MS Figure 1.



a) Phytic acid (b) phytate

Figure 1: Phytic acid and phytate structure [22, 25]

While other studies have shown that the phytate / iron molar ratio greater than 1, indicates a low iron bioavailability and that the phytate / zinc molar ratio greater than 15, is considered to be associated with reduced zinc absorption and a negative zinc balance [26]. Therefore, determination of phytate / mineral molar ratios would be interesting to measure the degree of inhibition of the latter by phytic acid and predict the availability of minerals in a food. Thus, these various results show that a high consumption of phytates can have harmful consequences on human health. Among these consequences, there are mineral deficiencies that most often affect women of childbearing age, children and people suffering from chronic blood loss. For example, in children and adolescents, iron deficiency is associated with decreased school performance [27].

To avoid these negative consequences of phytates, it is therefore important to put in place means to reduce their toxicity. To know the degree of toxicity or inhibition of phytates in a food, a determination of phytate / mineral molar ratios would be a good way.

Alpha-Galactosides

They are oligosides present in legumes seeds containing galactose whose osidic bonds are not hydrolysable by the digestive enzymes of human species [28, 29]. They are unreducing, low molecular weight sugars, soluble in water and hydro alcoholic solutions [30]. The most common oligosides are raffinose and stachyose Figure 2. Thus, the presence of alpha-galactosides in foods appears to depend on species and genotype [31, 32]. In cowpea seeds, the alpha-galactoside content varies

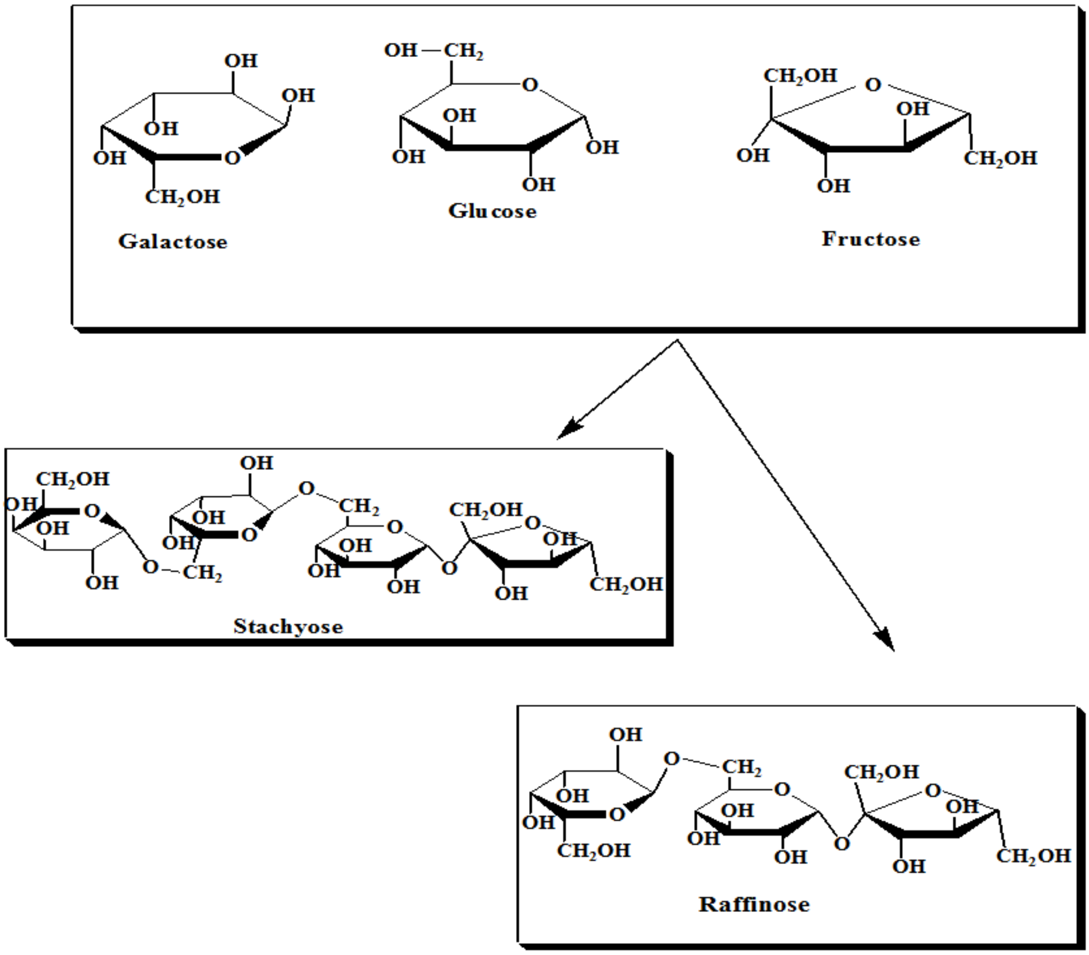


Figure 2: Chemical structure of raffinose and stachyose [30]

between 3.7 and 5.9% [33]. In different pea varieties, the content alpha-galactoside found varies between 2.3 and 9.6% [32, 34, 35]. However, lower levels of total alpha-galactosides (rafinose and stachyose) of 2.4% are found in lentil seeds [36].

These slightly different levels can be attributed to the difference in species between these two legumes. Alpha-galactosides are indigestible by the human body. They are not digested by humans because the intestinal mucosa is devoid of the hydrolytic enzyme α-galactosidase and these sugars themselves are unable to cross the intestinal wall [37, 38]. However, the microflora present in the large intestine is able to absorb them. Their absorption thus leads to the development of gases such as methane, hydrogen and carbon dioxide [38]. However, these gases are factors of flatulence for the human body. The major problem of alpha-galactosides is therefore their flatulence effect. The accumulation of flatulence in the intestinal tract causes discomfort, abdominal rumbling, cramps, pain and diarrhea. This phenomenon affecting certain individuals can be avoided if the levels are lowered. However, a thorough study is needed to determine threshold levels that can negatively affect the body.

Tannins

These are phenolic compounds of various structures that have the property of precipitating proteins. These are often polymerized compounds that give molecules of molecular weight ranging from 500 to 3000 Da [39].

The tannins are subdivided into two categories according to their structure: hydrolysable and non-hydrolyzable tannins.

1.Hydrolyzable Tannins

These tannins, also called tannoids, belong to the family of glycosides. They are polyesters of carbohydrates and gallic acid and its derivatives such as digallic acid [39, 40].

2. Condensed Tannins

They are flavanolic polymers, consisting of flavan-3-ol units linked together by carbon-carbon bonds, most often C4-C8 or C4-C6 Figure 3. Condensed tannins are often classified as flavonoids because of their similar structure. They do not contain sugars in their molecules and tend to polymerize by giving insoluble red

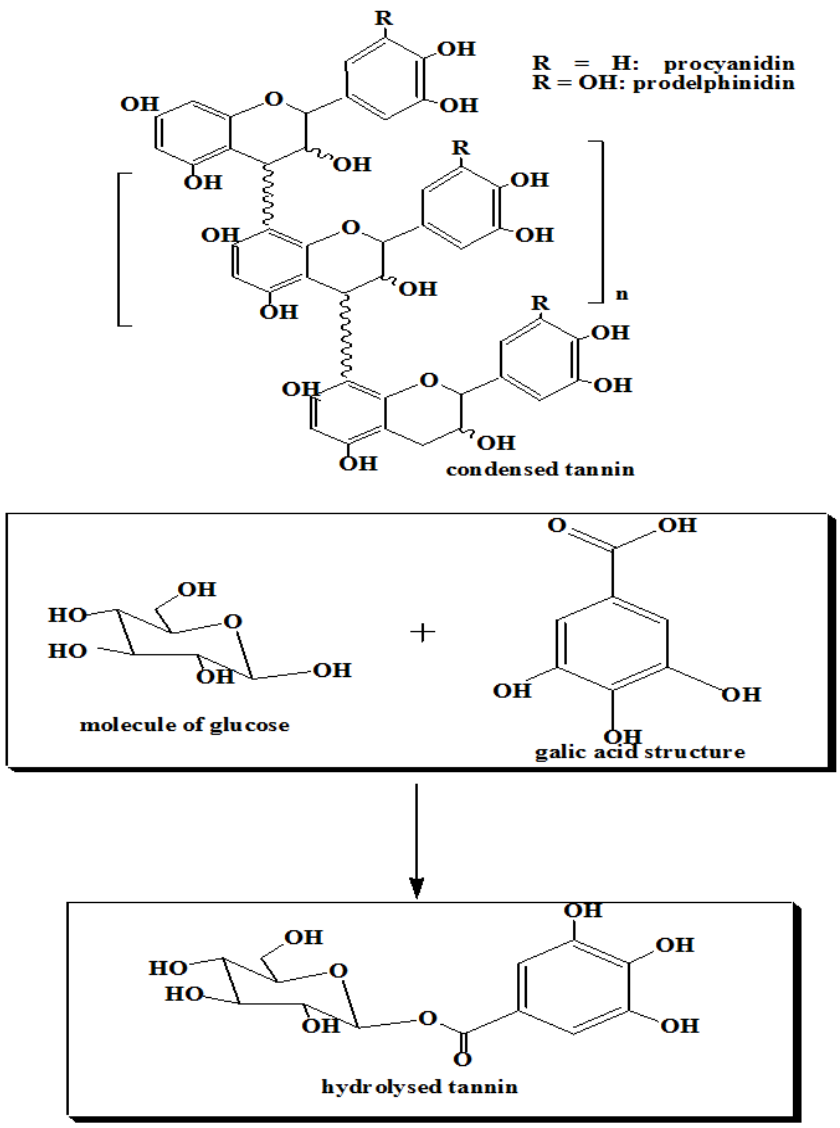


Figure 3: Structure of condensed and hydrolysed tannins [41, 42]

products [39, 41]. It is these condensed tannins which are found in grains cereals and legumes.

Legume seeds are characterized by a condensed tannin content of 0.75 to 4.78% DM [6]. Condensed tannins can be used as antioxidant additives in fatty foods [43]. However, they have the disadvantage of binding to proteins, carbohydrates and minerals in seeds, reducing their nutritional value [44].

In cowpea, the tannin content can reach up to 260 mg / 100gMS depending on the variety and color of the seeds [45]. This content is confirmed by the work of who found 270 mg / 100g, 680mg / 100g, 110mg / 100g and 820mg / 100g tannins in 4 cowpea varieties respectively [14]. In view of these results, it is clear that tannins are significantly present in cowpea seeds. They can therefore prevent the availability of certain nutritional elements such as minerals and proteins, and this, thanks to their ability to form complexes with these elements thus blocking their absorption.

Antinutritional molecules therefore hinder efforts to combat malnutrition, micronutrient deficiency and food security. It is therefore necessary to propagate abatement techniques while avoiding too much nutrient loss.

Processes for Reducing Anti nutritional Factors

To improve the nutritional quality of cowpea seeds by limiting the negative effects of ANFs, different strategies can be adopted.

Indeed, there are several methods of treating seeds including soaking, germination, fermentation, extrusion, pre-cooking with steam, etc.

Soaking

Traditionally, this operation was aimed at reducing the cooking time. This is an operation to hydrate the seeds by facilitating the diffusion and dissolution anti nutrients to the water phase. Soaking facilitates digestion and improves the absorption of contain nutrients by body [46]. The effect of soaking on ANFs content depends on its duration. Normal soaking requires an average of 8 to 12 hours at room temperature [16]. Table 1 gives some dipping processes carried out in different types of grains. A soak of 24 hours allows a significant reduction of its phytate content.

Table 1: Different soaking processes for reducing anti nutritional factors

Seeds Types	Soaking Solution Types	Ratio (Weigh : Volume)	Soaking Times / Soaking Temperature	Reduction Rate	References
Chick Pea	Solution (water + 0,01N NaOH + 0,01N HCl)	1 : 5	-----/-------	-----/------	(Haileslassie and al., 2016) [49]
Beans	Tap water	1 : 5	12 hours /----	13.04 %, 20.82 % for tannins	(Khandelwal and al., 2010) [50]
Cereal Grains (Millet, Sorghum And Maize, Rice)	Mineral water (Evian)	1 : 3	24 hours/ 30°C	28 % of phytates	(Lestienne and al., 2005) [24]
Bean	Distilled water	1 : 3	----/----	------/-------	(Piecyk and al., 2012) [51]
Red Bean	water (pH= 6,9) 0,05% Na_2CO_3, PH= 8,2 solution	1 : 3	12hours/ ambient	40, 41 and 45 % of alpha-galactoside	(Shimelis and Rakshit, 2007) [52]
Cowpea	Mineral water (Evian)	1 : 5	24 hours/ 30°C	Increase (-11.62%)	(Lestienne and al., 2005) [24]
Cowpea	Distilled water Solution of 0,02% Na_2CO_3; pH= 8,3)	1 : 10	2 , 4 et 6 hours/-------	10.81%, 19.60% for phytates and 22.22%, 32.92% for tannins	(Avanza and al., 2013) [14]

The reduction rates are 17% for rice grains, 21% for maize grains, 23% for soybeans and 28% for millet [24]. According to the results of a study on the evolution of anti nutritional factors of two cowpea seed varieties, soking in 24 hours resulted in a loss of 8.4% in phytates [47]. This loss of phytate levels has been confirmed or even exceeded by the studies of [48]. The latter obtained a reduction in the phytate content of 22.4 and 23.7% on two varieties of cowpea after 24 hours of soaking. The simple soaking of cowpea reduces the stachyose content by 30% [33]. These FAN reductions, which vary differently, can be attributed to their difference in water solubility. However, other dipping solutions are experimented such as the use of sodium bicarbonate.

Addition of sodium bicarbonate ($NaHCO_3$) to the soaking water can lead to significant reductions in ANF levels. Indeed, a 12 hours soaking with addition of sodium bicarbonate resulted in reductions of 41, 45 and 40% in total alpha-galactoside (stachyose and raffinose) in three varieties of red beans respectively [52].

These results show that there is a difference in the contents of reduced ANFs according to the soaking solution used. The addition of sodium bicarbonate caused a greater reduction compared to soaking with simple water. Thus, the sharp reduction observed during the soaking with addition of sodium bicarbonate is due to the tenderness of the seeds facilitating the rapid dissolution of these factors in the soaking solution. These studies show that soaking is a good way to reduce ANFs in legumes such as cowpea. Thus, the loss of these ANFs during soaking of the seeds can be explained differently.

Regarding tannins, classified as polyphenols, are located at the level of the teguments and their loss during soaking of the seeds is attributed to its effect of creating an ionic environment [14, 53]. The modified ionic environment could in turn modify the permeability of the integument, thus allowing larger and faster losses.

In addition, the decrease in phytic acid during this soaking process is attributed to its phytase catalyzed hydrolysis [14]. Thus this hydrolysis of the phytate molecule causes a release of minerals that become available for intestinal absorption.

However, it is important to note that during soaking, there is also a loss of certain nutritional elements such as minerals. It has shown by that cowpea seeds containing 6.60 mg / 100 g DM in iron are found with a content of 5.68 mg / 100 g MS or 13.94% loss after a 24h soak [24]. According to, the minerals (potassium, calcium, magnesium and iron) whose losses have been observed in the seeds are found in the soaking water [33].

It is therefore important to recommend dipping food as a method of ANF control while controlling the duration of the operation to avoid large losses of minerals and water-soluble vitamins.

Sprouting

By definition, germination is a process that incorporates events beginning with the absorption of water by the mature dry seed and ending with the protrusion of the radicle or more generally of a portion of the embryo through the envelopes of the seed [54].

Germination is often used to improve the concentration and bioavailability of nutrients in foods [55, 56, 57].

It improves the nutritional value of cereals and legumes by increasing protein digestibility, essential amino acid content and vitamins, while decreasing some anti nutritional factors [58]. Thus, during germination, important changes occur between the seeds and the culture medium. Changes include among others to the secretion of ions, oxygen and enzymes and a wide range of primary and secondary carbon-containing metabolites [59].

Germination can cause phytate hydrolysis and decrease their inhibitory effects on mineral uptake. It lasts a few days and can be initiated by a few simple steps namely rinsing the seeds to remove all the impurities, soaking the seeds in the water. Table 2 gives a summary of the conditions used for germination for different types of cereals and pulses. Germination results in 37 to 81% reduction of phytate in different types of cereals and legumes [60].

Table 2: Conditions used for germination of different types of kernels

Seeds Types	Ratio Seed : Soaking Solution(W/V)	Times /Temperatures/ Nature Of Soaking Solutions	Duration Of Germination	Reduction Rate	References
Millet, Sorghum	-----	24 hours/25°C/-----	7 days	------	(Ochanda and al., 2010) [61]
Amaranth	-----	Night/----/ Distilled water + 0,2% formaldehyde solution	72 hours	34,66% of tannins	(Olawoye and Gbadamosi, 2017) [62]
Beans (Green Beans / Mung Beans)	1 :5	12hours/ambiente/ Tap water	24 hours	43% of tannins	(Khandelwal and al., 2010) [50]
Red Beans	1 : 5	12 hours/25°C/ Distilled water + 0.01% (w/v) d'eau de javel	4 days	76% of tannin ; 92% of phytic acid	(Shimelis and Rakshit, 2007) [52]

The 4-day sprouting of three bean varieties resulted in a gradual reduction of phytic acid levels from 23.51 to 1.88, from 24.06 to 5.06 and from 17.34 to 0.69 mg / g are losses of 92.00%, 79.76% and 96.02% respectively [52]. This technique is an effective method to reduce phytate levels, but its effectiveness is variable depending on the germinated material, the germination conditions and especially the germination time.

Moreover, the efficiency of the germination is maximum if the amount of enzymes capable to hydrolyze phytates is high and if the germination conditions are favorable to the activity of its enzymes. Germination also causes large reductions in phenolic compound contents ranging from 9 to 56% and particularly in tannins from 33 to 72% [6].

In a study of the evolution of anti nutritional factors of two varieties of cowpea seed, "voanembafotsy" and "voanemba mena", it was found that after 48 to 96 hours of germination, the phytate content cowpea is reduced by 48.8 and 81.8% respectively [47].

According to, after 96 hours of germination, the phytate content of the mara variety decreased by 41.5% under laboratory conditions [48]. Other studies have shown that the raffinose content decreases steadily with increasing germination time.

For a period of 5 days of germination, the raffinose content decreased steadily by 67% [63]. While found raffinose reductions of 38 and 62% for 24 h and 48 h germination respectively [52]. For the same germination times, the stachyose content was reduced by 53 and 92%, respectively [52]. This decrease in phytates and alpha-galactoside is due to the presence of specific enzymes, phytase and alpha-galactosidases. In fact, the different phytases present in the seeds act during seed germination [64]. A study comparing the effect of pressure cooking, dipping and of germination showed that the greatest reduction in polyphenol and tannin content was achieved by germination, followed by dipping and pressure cooking [50]. The different results observed during germination confirm its effectiveness in reducing FANs. Thus, germination times varying between 48 and 72 h would be sufficient to significantly reduce the FAN content in legume seeds.

Fermentation

Fermentation is one of the effective, inexpensive and nutritionally beneficial domestic processes [65]. Fermentation not only improves food safety and shelf life, naturally preserving food and also improves nutritional value [61].

This is a treatment technique that starts with the hydrolysis of starch by the action of enzymes. In general, lactic acid bacteria are essential in fermentations for the production of metabolites, degradation of cyanogenic glucosides, production of enzymes, probiotic properties and the production of many other molecules [66]. The reduction of FAN levels during fermentation is attributed to the activity of fermentative microorganisms [67]. Fermentation leads to a decrease in alpha - galactosides and phytates and has a positive effect on the availability of iron and other minerals [68, 69]. It promotes optimal pH for the enzymatic degradation of phytates [69].

It reduces the levels of some anti nutritional factors, particularly phytates and α-galactosides, in cereals and legumes, for millet, it reduces phytate and raffinose content by 75% and 83% respectively [70, 71]. Fermentation is therefore a microbial and enzymatic method of treatment which not only extends the shelf life of foods but leads to a significant reduction in FAN levels. This reduction role is indeed made possible by the lowering of the pH. Compared with soaking, the reduction rate obtained by fermentation is more significant; this leads to the conclusion that the use of fermentation for the treatment of seeds legumes and particularly cowpea would be a good asset of the nutritional point.

Extrusion Cooking

Extruders whose use for food processing for the first time dates back to 1869 and has led to a widespread development of extrusion cooking [70]. The extrusion cooking technique is a process that combines thermal and mechanical processes to produce a precooked product. This technique reduces ANFs by increasing the bioavailability of minerals [72]. It thus makes it possible to reduce phytates by approximately 30% [73]. It was found by reductions in phytic acid of 26.73% and 20.75% in extruded seeds of beans (Vicia faba L.) and green beans (Phaseolus vulgaris) respectively[74]. Studies have shown that during the extrusion cooking process some phytic acid molecules are hydrolysed to inositol penta-, tetra- and triphosphates [74].

However, the extrusion temperature and the moisture of the product to be extruded play an important role in the reduction of ANFs. Thus, a 55.83% reduction in phytic acid was observed during extrusion cooking of rice bran at a temperature of 140 ° C and 20% humidity [75]. A lower moisture content during extrusion results in a lower degradation of the phytic acid of grain brans. According to at 115 °C the average values of phytic acid in extruded rice brans were 19.92, 18.63 and 17.35 mg / g at 14, 17 and 20% humidity respectively [75]. The observation of these results makes it possible to remember that the extrusion temperature is a factor that can significantly influence the ANFs content compared to the moisture of the extruded product. Thus, 140°C temperature and 20% humidity would be the most appropriate parameter pair for reducing ANFs in a food product. In addition, the technique of extrusion cooking compared to the other processes studied above would be preferable. Indeed, it makes available minerals without destroying them while other processes such as soaking and germination cause a diffusion of these minerals in the soaking water.

Steam Pre-Cooking

Steam pre - cooking is a unit operation used for the processing of certain food products, in this case rice and millet. It is applied to enhance the nutritional quality of rice [76]. Studies have shown that this process reduces FAN levels in legume and cereal seeds.

In fact, steam pre – cooking resulted in a 52% reduction in phytate content of beans, 56.5% in sunflower and 47.9% in rice grains [77]. In the production of "moin-moin" (steamed cowpea paste containing seasonings), there was a decrease in phytate contents between 7.8-14.0% and tannins of 19.6-24. 7% [78]. This decrease in phytate during heat treatment would be the result of their degradation by heat. This is due to the thermolabile nature of phytic acid [79].

For tannins, they are localized at the level of grain teguments and are destroyed during the heat treatment process [80]. In this thermal process, the temperature and the treatment time are good optimization parameters. Reductions in average phytic acid content from 38.14 mg / g for untreated cereal brans at 18.72 mg / g for cereal brans subjected to steam pre-cooking at 100 ° C for 25 minutes, 18.08 mg / g at 110 ° C for 25 minutes and 18.74 mg / g at 115 ° C for 25 minutes were recorded [81]. These recordings showed that the maximum reduction (52.60%) of the phytic acid content was obtained at 110 ° C for 25 minutes.

Thus, in the case of cowpea, similar losses in ANFs could be considered because of the heat labile nature of some of them or their location in teguments whose heat facilitates their destruction. A combination of this treatment technique with the other processes could give better reductions in ANFs while retaining nutritional and organoleptic qualities of the final product.

Conclusion

This literature review has revealed that there are various methods of reducing anti nutritional factors. Reduction techniques such as soaking, germination, fermentation, extrusion cooking and steam pre-cooking have been found to be often used methods. Thus, the study showed that a significant reduction in ANFs greater than 50% is obtained by germination, fermentation, extrusion cooking and pre - cooking with steam. However, the steaming technique is rarely used, especially in the case of cowpea. This allows us to think that the combination of this process with other techniques could be a good track for reducing ANFs in food and especially cowpea.

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