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Enzymatic activities, digestibility and functional properties of germinated white beans (*Phaseolus vulgaris* L.) seeds from Daloa (Côte d'Ivoire)

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Abstract

The objective of the present study was to evaluate enzymatic activities, functional properties and starch digestibility of the flour of germinated seeds of white bean (*Phaseolus vulgaris* L.) after three days of germination. Results revalated that germination induces significantly ($p \leq 0.05$) activation synthesis of hydrolytic enzymes that make nutrients available for plant growth and development. Thus, the amylase, cellulose, α and β glucosidase activities increase significantly during germination in the different samples of bean flour. However, a significant decrease in the activities of α and β galactosidase was observed in the sprouted bean seeds. Therefore, germination is an effective processing method for improving enzymatic activities, boosting the level and digestibility and improving the functional properties of legume, particularly beans. The water and oil absorption capacity, foaming capacities and emulsification properties of bean flours increase significantly after the bean seeds germinate. But germination decreased bulk density, foam stability and sedimentation value of the white bean seed flour.

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Introduction

Vegetable (cereals and legumes) seeds constitute an essential part of the human diet as they are excellent sources of proteins, minerals, vitamins and bioactive compounds (Magalhães *et al.*, 2017). Legumes for exemple play an important role in the agriculture and diet of many developing countries and are a major source of dietary nutrients for many people (Ghavidel and Prakash, 2007 ; Çakir *et al.,* 2019). They are a good and inexpensive source of energy, dietary proteins, carbohydrates, vitamins and minerals.

The white bean (*Phaseolus vulgaris* L.) for example, is the most produced and consumed legume in the world (FAOSTAT, 2019). Thus, it occupies an important place in human nutrition in the regions of Africa and particularly in Côte d'Ivoire (Njoroge *et al.*, 2015). In addition, it 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, white bean possesses low starch digestibility that has been shown to cause a higher loss of energy in humans (Gnanwa *et al.*, 2021). And the presence of antinutritional compounds can affect the protein and starch digestibility as well as other nutrients (Lemmens *et al.*, 2019). Therefore, processing methods such as fermentation and germination that expose the starch granules and protein matrix to digestion may help to overcome the digestibility problem. Germination is a processing method that enhances enzymes activities, the nutritional and functional properties of grains as well as their digestibility (Anaemene and Fadupin, 2020). On the other hand, germination is inexpensive technology which results in structural modification and synthesis of new enzymes with high biological activity, increased nutritional value and stability of seeds (Singh and Sharma, 2017).

Thus, flour prepared from germinated seeds are suitable for the preparation of wide consumed speciality foods and value added products across the globe (Xu *et al.*, 2017). Thus, the changes in the chemical composition influence the spatial arrangements of molecules and therefore affect the functional properties of flour (Siddiq *et al.*, 2009). Functional properties are the physiochemical characteristics that interact with the chemical properties of food components (Sibian *et al.*, 2017). Furthermore, knowledge of biochemical and functional properties of flours prepared from germinated seeds are desirable for their enhanced utilization (Chinma *et al.*, 2017 ; Singh *et al.*, 2017). This is why the present study investigated the effect of germination on enzymatic activities, digestibility and functional properties of white bean seed flour rom Daloa area for application in food preparations.

Materials and methods

Raw material

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

Fig. 1. Ungerminated white bean (*Phaseoulus vulgaris* L.).

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 liters 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. The seeds germinated for three days and were prepared for the flours and assay for enzymatic activities.

Flour production

The bean seed samples were oven dried for 48 hours at 65°C. They were then ground in a MOULINEX brand mixer to obtain a flour. This flour was stored in previously dried jars for possible analysis (Fig. 3).

Fig. 2. Germinated white bean seeds (*Phaseoulus vulgaris* L.).

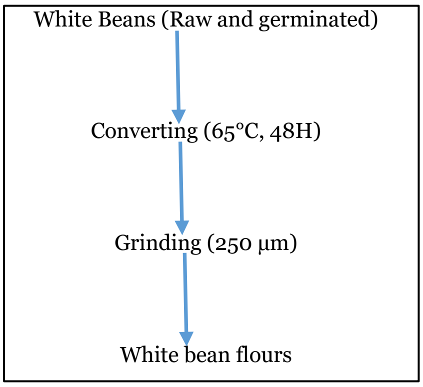

Fig. 3. Flow diagram of the process for producing white bean flour.

Preparation of the crude enzymatic extract of bean seeds

Fifteen grams (15g) of raw and sprouted bean seeds are ground in 40ml of 100mM sodium acetate buffer pH 5.0 containing 0.9% (w/v) of sodium chloride using a porcelain mortar and pestle. The ground material is centrifuged at 4000 revolutions per minute for 30 minutes. The collected supernatant constitutes the crude enzymatic extract.

Preparation of the enzymatic extract of digestive snail juice digestive juice

The digestive juice of the snail *Archachatina marginata* is collected according to the method described by Colas (1977).

Determination of heterosidic activities

The reaction medium consists of 150µl of 100mM sodium acetate buffer, pH 5.0; to which are added 25µl of the enzymatic preparation and 75µl of p-nitrophenyl glycoside substrate (5mM). This medium is incubated and the reaction is stopped as above, and the quantity of pNP released is determined using a spectrophotometer (SPECTRONIC R GENESYS TM 5) at 410nm. The optical densities obtained are also converted into micromoles of p-nitrophenol released per minute and per milligram of protein or as a percentage of activity as described under standard conditions.

Determination of polysaccharide activities

The reducing sugars released during the enzymatic hydrolysis of polysaccharides (inulin, soluble starch, xylan and carboxymethylcellulose) were determined according to the method of Bernfeld (1955) using 3,5 dinitrosalycilic acid (DNS).

Starch digestibility

The hydrolysis of the starch in vitro by an amylase of the digestive snail juice (20µl of enzymes per g of starch) was followed for 120 min in a water bath at 37°C. The sugars released were quantified by the method of Bernfeld (1955) using 3,5 dinitrosalycilic acid (DNS).

Functional properties of samples

Water absorption capacity and solubility index in water

The water absorption capacity (WAC) of ungerminated and germinated bean flour were determined according to the method of Claver *et al.* (2010). Distilled water (10ml) was added to 1g of sample, and the mixture was mixed thoroughly using a vortex mixer for 30 min and centrifuge at 4000 rpm for 15 min. The mass of water absorbed was expressed as g/g starch on a dry weight basis. The water absorption capacity and solubility index in water were expressed as percentage increase of the sample weight.

Oil Absorption Capacity

Oil absorption capacity of the flour samples was determined by the centrifugal method elicited by Eke et Akobundu (1993) with slight modifications. One gram of sample was mixed with 10ml of oil, the mixture was allowed to stand for 30 min at room temperature, centrifuged at 4000 g for 15 min and the oil that separated was carefully decanted and the tubes were allowed to drain at 45° angle for 10 min and then weighed. Oil absorption was expressed as percentage increase of the sample. And the hydrophilic-lipophilic ratio (HLR) as defined by Njintang *et al.* (2001).

Foaming capacity and stability

The procedure of Coffman and Garcia (1977) was used. Three grams of flour sample and 50ml distilled water were mixed in a Braun blender at room temperature. The suspension was mixed and shaken for 5 minutes at 1600rpm. The content along with the foam was poured into a 100ml graduated measuring cylinder. The total volume was recorded after 30 seconds. Then the content was allowed to stand at room temperature for 30 minutes and the volume of foam only was recorded.

Bulk Density

Bulk density of the starch was determined according to the method of Musa *et al.* (2008). Flour (20g) was weighed into a 50ml measuring cylinder and the volume occupied was measured and recorded. The cylinder was gently tapped on the bench top 10 times from a height of 5cm. The bulk density was calculated as weight per unit volume of sample.

Dispersibility

The dispersibility of white beans (ungerminated and germinated) flours were measured according to the method of Mora-Escobedo *et al.* (1991). One gram of the flour was dispersed in distilled water in a 50ml stoppered measuring cylinder. Then distilled water was added to reach a volume of 30ml, the mixture was stirred vigorously and allowed to settle for 20 min, the volume of settled particles was subtracted from 30 and multiplied by 100 and reported as percentage dispersibility.

Emulsification properties

Emulsification capacity

Emulsification capacity was calculated by the modified method of Yasumatsu *et al.* (1972). 2.0g of sample was taken and blended with 25ml distilled water. Corn oil was taken in burette and added to the mixture with continuous blending until the break point was reached (i.e. separation of oil from aqueous phase). The emulsification capacity (EC) was expressed as ml of oil emulsified by 1.0g of the sample.

Emulsification activity

Emulsification activity was calculated by the method of Yasumatsu *et al.* (1972). 3.5g of flour sample was homogenized in 50ml water and re-homogenized by adding 50ml corn oil for 90s. Emulsion was then transferred to two centrifuge tubes equally and centrifuged for 5 min at 1100×g.

Emulsion stability

Emulsion stability was calculated using the above sample after calculating emulsifying activity (Yasumatsu *et al.*, 1972). Sample was heated at 85 ± 2°C, for 15 minutes and then centrifuged at 1100g for 5 min. Emulsion stability was expressed in percentage as the emulsifying activity remaining after heating using formula for the calculation of emulsion activity (%).

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 $P \leq 0.05$.

Results and discussion

Enzymatic activities

In terms of enzymatic activities, a significant increase ($p < 0.05$) was observed in amylase, cellulase and glucosidase activities while a significant decrease ($p < 0.05$) was observed in galactosidase activities after germination of bean seeds (Table 1). Indeed, Di Stefano *et al.* (2019) reported that sprouting increased α-glucosidase activity in chickpeas, broad beans and yellow peas. In addition, an appreciable increase in amylase activity was observed in beans, cowpeas, lentils and chickpeas at 72 hours of germination (Ghavidel *et al.*, 2011). On the other hand, germination leads to a significant increase in α-amylase activity in cowpea, as this enzyme is needed to hydrolyze starch into metabolizable sugars, which in turn provide energy for the growth of roots and leaves shoots (Kaczmarska *et al.*, 2017). According to Nkhata *et al.* (2018) evidence indicates that sprouted foods are high in nutrients compared to non-sprouted foods due to the activation of endogenous enzymes that degrade anti-nutritional factors. Storage compounds in dry seeds (α-galactosides) decreased after germination as they were hydrolyzed to glucose and fructose, compounds that can serve as an energy source for the new plant (Vidal-Valverde *et al.*, 2002). Garduza-Acosta *et al.* (2020) reported in their work on Crotalaria and Lupinus seeds (legumes) that once most of the seeds have germinated, α-galactosidase activity decreases. These observations are entirely in agreement with the results obtained with the sprouted bean seeds. This would reduce the problems of flatulence and this is the reason why special attention is paid to the level of α-galactosidase activity during germination. Therefore, sprouted bean seeds could be eaten as germination corrects gas problems.

Table 1. Enzymatic activity of bean (ungerminated and germinated) flours.

Enzyme Activities (UI/mg of Protein)	Ungerminated beans	Germinated beans
α-glu	0.27 ± 0.02^{a}	0.69 ± 0.02^{b}
β-glu	0.12 ± 0.01^{a}	0.35 ± 0.03^{b}
α-gal	2.55 ± 0.30^{b}	0.61 ± 0.03^{a}
β-gal	1.68 ± 0.25^{a}	0.44 ± 0.04^{b}
Amylase	0.26 ± 0.03^{a}	0.52 ± 0.04^{b}
Cellulase	0.18 ± 0.01^{a}	0.36 ± 0.03^{b}

*n=3, Results are expressed as mean values ± standard deviations. Means in a row with different superscripts are significantly different (P<0.05)

Digestibility

The results obtained in this study show that germination induces an increase in the digestibility of starch in bean flours (Fig. 4). These results are in agreement with those found by Lemmens *et al.* (2019). These authors have shown that the digestibility of starch increases following germination time. This has been mainly attributed to the breakdown of anti-nutrients such as amylase inhibitors, phytic acid and polyphenols which inhibit the action of α-amylase (Lemmens *et al.*, 2019). Sprouted legumes are generally better digestible due to their enzymatically damaged starch granules, thinner cell walls, and higher content of readily available sugar (Yan *et al.*, 2010). In fact, the *in vitro* digestibility of starch from sprouted seeds is due to the degradation of the starch chain by amylolytic enzymes; which makes the chains easily digestible. Germination also improves the biological value of proteins. Thus, Ghavidel and Prakash (2007) have showed that the in vitro digestibility of protein, crucial in determining the protein quality of food, was increased by a range of 14% – 18% after germination of green gram, cowpea, lentil, and chickpea. Therefore, the present bean sprouts would be suitable for the production of foods for infants and the elderly.

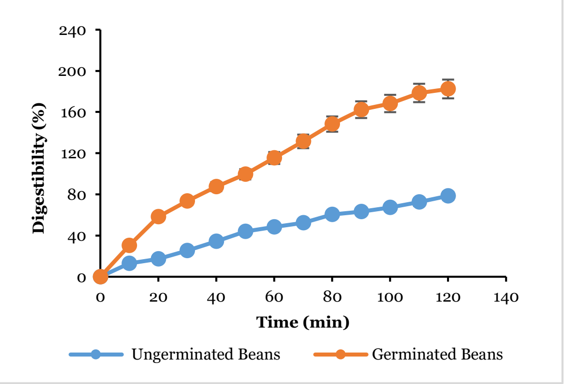

Fig. 4. Evolution of the digestibility in vitro of beans flour over time.

Functional Properties

The results show that germination increased the water absorption capacity of white bean (Table 2). Water absorption capacity (WAC) of raw white bean was 164.00 ± 1.00% water absorbed and increased to 250.22 ± 3.02% water absorbed as a result of germination. This increase in the water absorption capacity of germinated flour might be attributed to breakdown of polysaccharides and increase in the sugar and protein content. The high water absorption capacity (Table 2) of this sprouted bean meal could be explained by its low fat content. Indeed, according to Karim *et al.* (2020), the presence of lipids in large quantities in a flour would reduce the binding capacity of water to particular substances, thus limiting the absorption capacity of water. In addition, this increase in water uptake may be due to a change in the protein of legume seeds, which could lead to more water uptake sites (El-Adawy *et al.*, 2003).

The water absorption capacity of the present germinated bean meal is higher than those reported by Du *et al.* (2013) on pulse flours (117 to 189%). High WAC of flours suggests that the flours can be used in formulation of some foods such as sausage, dough, processed cheese and bakery products.

Table 2. Functional properties from ungerminated and germinated white beans.

Functional Parameters	Values	
	Ungerminated beans	Germinated beans
Water absorption capacity (%)	164.00 ± 1.00^{a}	250.22 ± 3.02^{b}
Solubility Index in Water (%)	22.36 ± 0.84^{a}	30.09 ± 1.25^{ab}
Bulk density (g/mL)	0.90 ± 0.01^{b}	0.75 ± 0.02^{a}
Emulsification activity	28.83 ± 0.10^{a}	37.20 ± 0.40^{b}
Emulsification capacity (mL Oil/g sample)	23.08 ± 0.03^{a}	28.72 ± 0.04^{b}
Emulsification stability	18.37 ± 0.82^{a}	24.45 ± 1.62^{b}
Foaming capacity	20.30 ± 0.75^{a}	25.49 ± 0.27^{b}
Sedimentation value (mL)	38.63 ± 1.20^{b}	29.67 ± 2.33^{a}

*n=3, Results are expressed as mean values ± standard deviations. Means in a row with different superscripts are significantly different ($P<0.05$)

Bulk density was reported lower in the germinated white bean flour sample and observed higher in raw grain flour. This change could be attributed to decrease in mass per unit volume as a result of germination. Germination lowers the bulk density of white bean flour. Decrease in bulk density with germination might be due to lowering of heaviness and dispensability of flour particles. Similar observations have also previously been reported by Udensi and Okoronkwo (2006).

Foaming capacity corresponds to the ability of proteins to form foam and its surface activities. Germination in white bean caused higher surface activity of protein and thus resulted in increase in the foaming capacity (Njintang and Mbofung, 2006). Foaming capacity of white bean flour varied from 20.30 ± 0.75 to 25.49 ± 0.27%.

Sedimentation value of germinated white bean flour was lowered as compared to raw white bean flour. Sedimentation value ranged from 38.63 ± 1.20 to 29.67 ± 2.33ml. Decrease in the sedimentation value might be attributed to the breakdown of particularly gluten protein as a result of protease activity during germination.

Emulsification properties like emulsification activity, emulsification capacity and emulsification stability varied significantly as a result of germination. Emulsification activity percentage of raw white bean flour was reported as 28.83 ± 0.10%, which increased to 37.20 ± 0.40% as a result of germination. Emulsification capacity of germinated white bean flour was high and reported as 28.72 ± 0.04 (ml oil/g), whereas emulsification capacity of raw sample was 23.08 ± 0.03 (ml oil/g). Emulsification stability of white bean flour varied from 18.37 ± 0.82 to 24.45 ± 1.62 as a result of germination. Enhancement in the emulsification properties of white bean flour after germination could be attributed to the interaction of fats and protein content (Makri *et al.*, 2005).

Oil absorption capacity (OAC)

Oil absorption capacity was determined to measure the ability of the flour protein to physically (Table 3) bind fat by capillary attraction. Oil absorption capacity is important since oil acts as flavour retainer and increases the palatability of foods (Kinsella, 1976). Oil absorption capacity was also reported high in white bean flour of germinated grains. The results obtained show that the OAC ranged between 120.29 ± 1.98 to 154.00 ± 1.00% and between 124.54 ± 1.56 to 191.37 ± 1.78% of raw and germinated white bean flour respectively with different oils. The highest values were found with red oil (154.00 ± 1.00 and 191.37 ± 1.78%) and the lowest with olive oil (120.29 ± 1.98 and 124.54 ± 1.56%) respectively of germinated and raw white bean flour. Chinma *et al.* (2009) reported the similar observations in tiger nut flour as a result of germination. The high oil retentions (Table 3) which were observed in germinated bean flour would be due to availability of lipophilic groups and to the ability of the proteins of this flour to retain oil (Suresh and Samsher, 2013).

Therefore, the high oil absorption capacity of the present germinated bean meal makes it suitable for facilitating the improvement of flavor and mouthfeel when used in food preparations (Appiah *et al.*, 2011). Thus, the major chemical component affecting OAC is protein which is composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form hydrophobic interaction with hydrocarbon chains of lipids (Ratnawati *et al.*, 2019). The oil absorption capacities obtained in the present study are superior to those mentioned by Aguemon *et al.* (2019) on ackea (*Blighia sapida*) seed meal with values of 107 ± 0.03 and 96.45 ± 0.04% for red oil and oliveoil respectively. However, the oil retentions obtained are lower than those reported by Ratnawati *et al.* (2019) on certain legume flours, the proportions of which varied from 303 to 360%.

Table 3. Oil Absorption Capacity and Hydrophilic-Lipophilic Ratio from ungerminated and germinated white beans.

Different Oils	Values		Hydrophilic-Lipophilic Ratio (HLR)	
	Ungerminated Beans	Germinated Beans	Ungerminated Beans	Germinated Beans
Redoil	154.00 ± 1.00[a]	191.37 ± 1.78[b]	1.06	1.31
Refinedoil (Dinor)	131.44 ± 2.25[a]	140.42 ± 0.94[b]	1.25	1.78
Oliveoil	120.29 ± 1.98[a]	124.54 ± 1.56[b]	1.36	2.01

*n=3, Results are expressed as mean values ± standard deviations. Means in a row with different superscripts are significantly different (P<0.05)

Conclusion

Germination of white bean *Phaseolus vulgaris* L. seeds flour resulted in increased amylase, carbomethylcellulase, α and β-glucosidase activities but α and β-galactosidase ones decreased. The water and oil absorption capacities, foam capacity, emulsification properties of white bean seed flour increased significantly with increase in the germination time while bulk density, sedimentation value and foam stability decreased. Unlike ungerminated seeds, germinated bean kernels are more digestible with relatively high levels of good functional properties that may be useful in food systems where they can play many functional roles. For example, the water absorption capacity WAC and oil absorption OAC of white bean flour make it useful for various products that require water and oil retention for their textural integrity like oil retention capability helps retain flavor and provides good mouth feel. Therefore, the improved functional properties of the germinated white bean seed flour could be utilized in food systems where natural modified flour is required rather than chemically or thermally modified white bean seed flour.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ethical review: This study does not involve any human or animal testing.

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