



Assessing the Microbiological Level and the Incidence of Water-soaking on the Proximate Composition of two Cultivars of Cowpea (*Vigna unguiculata L.*) Grains Grown in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author RKN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MK managed the analyses of the study. Author KK performed the analysis in the laboratories and managed the literature searches. Author WAMAB managed the analyses of the study and revised the first draft. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study was undertaken to assess the influence of seed treatment (soaking in water) on nutritional and microbiological composition of two cowpea cultivars.

Place and Duration of Study: Laboratory of Food Biochemistry and Tropical Products Technology, and the laboratory of Biotechnology and Food Microbiology, University of Nangui Abrogoua, Abidjan, between October 2010 and December 2011.

Study Design: Method based on AOAC tests and AFNOR for microbiological analysis. A two-way analysis of variance and t-test were used.

Methodology: The proximate composition of soaked and non soaked cowpea grains was determined and microbiological (bacteriological and mycological) analysis of these grains

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was also performed.

Results: The major components were 28% and 26.25% protein, 48.35% and 47.99% carbohydrate, 41.66% and 40.05% starch for the RC (red cultivar) and WC (white cultivar) respectively. Lipids are less represented in the 2 cultivars (2.5%). There were significant reductions in the contents of the major components as a result of the treatment. Plain water soaking brought about a significant decrease in the proximate composition causing a mean reduction of 3.14% and 10.02% protein, 28.23% and 29.30% carbohydrate, 29.47% and 28.94% starch, 18.80% and 22.02 % energy for the RC and WC respectively. The mean decrease for mineral was 23.13% and 47.66% iron, 2.32% and 8.15% calcium, 9.30% and 2.10% phosphorus for the RC and WC respectively. In general the highest reduction was observed in the WC variety. Mean count (Log_{10} cfu/g) of total aerobic microflora, coliforms, mould and yeast were 6.29 and 6.43; 2.04 and 2.58; 4.41 and 4.78 for the RC and WC respectively. Five genera of mould were isolated: *Aspergillus*, *Mucor*, *Penicillium*, *Botrytis* and *Geotrichum*. The predominant fungi belonged to *Aspergillus* genus.

Conclusion: The cultivar types of cowpea and the preparation methods could affect the nutrient availability of this product. Cold water soaking has a great influence on the properties of cowpea grains.

Keywords: Cowpea; cultivar; water soaking; mould; proximate composition; soaking.

1. INTRODUCTION

Cowpeas (*Vigna unguiculata* [L] Walp) are regarded as popular and important indigenous African legume crops by many rural communities living in the less developed countries of tropical and subtropical Africa [1,2]. Cowpeas are important legumes in the diets of many people living in the northern area of Côte d'Ivoire. Cowpeas are more drought resistant than common beans. Drought resistance is one reason that makes cowpea such an important crop in many underdeveloped parts of the world. They are a good source of carbohydrates, vitamins, and protein, providing more than half of vegetable protein in human diets in some areas of the semiarid tropics [1,3]. Grain legumes such as cowpeas play an important role in the diets of many populations in protein-deficient countries [4]. Cowpea seeds are susceptible to fungal contamination when stored in poor conditions such as relative high humidity and high ambient temperatures [5]. It is also under these conditions that certain fungi may produce toxic secondary metabolites, namely mycotoxins. The ingestion of mycotoxins in contaminated agricultural products can lead to detrimental health problems for humans and farm animals [6].

Cooking cowpeas requires heating the grains with live steam at temperatures above 100°C [7]. Soaking the grains before cooking them helps in the removal of seed coat to shorten the cooking time, the energy used and allows an easier dehulling process. During the soaking process, the grains are hydrated to some extent and may lose some of the chemical and nutritional characteristics. This process (soaking, cooking) facilitates the dehulling of the seeds, but can cause nutrient leaching. The chemical components of the cowpeas influence their absorption properties and hence the processing time while the microbiological properties influence grain spoilage and the sanitary quality of the grains for the consuming public.

Limited researches have been published on the nutritional, functional and processing aspects of cowpeas grown in Côte d'Ivoire. Moreover, there is no available information on

the natural occurrence of microorganisms in dried cowpea grains. Though such study is limited in Côte d'Ivoire, a lot literatures are cited in other countries [8,9,10,11]. In Côte d'Ivoire, there is still a lack of information on cowpea seed-borne fungi and their role in disease development. Therefore, it seems important to evaluate the microbiological level of cowpea grains and determine the fungal profile. To our knowledge, this is the first study of its kind in this country. Thus, the present study was conducted to assess the influence of soaking in water treatment on nutritional and microbiological composition of two cowpea cultivars.

2. MATERIALS AND METHODS

2.1 Materials

Two cultivars of cowpea grains, Red and White cultivars (RC and WC) commonly consumed in Côte d'Ivoire and used for this study were obtained in Abidjan, Côte d'Ivoire at a marketplace known as «Adjamé Gouro». These two cultivars were identified at the floristic center of the University of Cocody, Abidjan as *Vigna unguiculata*, a climbing plant variety. Approximately, 6 kg of each cultivar grains per replicate were divided into 40 seed lots; a seed lot represents a sample of 150 g of grains. The grains were packed and transported the same day to the laboratory (Abobo-Adjamé, University). Immediately after, the grains were sorted manually to separate wholesome grains from insect-infested or broken damaged grains. The grains were stored in a refrigerator (5°C) until analyses.

2.2 Methods

2.2.1 Soaking procedure

A sample of 10 g of cowpeas was soaked in 400 mL tap water for 18 h at room temperature (30°C±2). The soaked grains were drained, dried on a paper towel, ground for 3 min in a coffee mill and stored in a polyethylene bag for proximate analysis. The yield was calculated by comparing the sample components prior to soaking.

2.2.2 Chemical analysis

Soaked and non soaked cowpea grains were analysed. The following composition characteristics were determined for each sample. The moisture content was determined by calculating the difference of weight before and after the desiccation of the moisture until constant weight; the ash fraction was determined by the incineration of the organic matter at 550°C [12], and the minerals by Atomic Absorption Spectrophotometer (Pelkin Elmer, model PE 3110, Norwalk, CT, USA) but phosphorus according to the method described by Tausky & Shorre [13]. Proteins were determined through the Kjeldahl method, and the fat through the Soxhlet extraction method (Unid Tecator, System HT2 1045, Sweden). The carbohydrate, starch and cellulose were determined by the cuprometric method of Bertrand and Thomas [14]. The pH of the samples were measured by the method described by Clawson and Taylor [15] using a calibrated pH meter (PHM85 Precision pH meter, Copenhagen, Danmark) on a mixture of 50g blended cowpea grains in 80 mL distilled water. The acidity was done according to the French Industrial Standard Authority and the Energetic density was calculated with the specific coefficients of Atwater & Rosa [16]. All the determinations were made in triplicate. The results were expressed on dry weight basis, and mean values were given.

2.2.3 Microbiological analysis

2.2.3.1 Mycological analysis of cowpea grains

Prior to plating, 50 g of each seed lot were surface disinfected in 1% sodium hypochlorite for 1 min (treated grains) [4]. The treated grains were intended to be used to determine contamination inside the grains. Fifty (50g) gram grains from the same seed lot were not surface sterilized (untreated grains) and were stirred for 5 min at room temperature in 10 mL of peptone water) then 0.1 mL of this suspension was spread-plated onto Dichloran Rose Bengal Chloramphenicol agar (DRBC). The treated grains were washed in 10 mL sterile distilled water to wash out the disinfectant and suspended into 10 mL of peptone water. Then, 0.1 mL of this suspension was spread-plated onto Dichloran Rose Bengal Chloramphenicol agar. The plates were incubated at $25 \pm 2^{\circ}\text{C}$ for 3 to 7 days. Fungal isolates were identified by culture appearance and microscopical observation using taxonomic keys [17,18], and recorded to determine the predominant types of moulds.

2.2.3.2 Bacteriological analysis of cowpea grains

The presence of microorganisms was enumerated using the agar plating method. To analyze the samples, the methods stated in the Compendium of Methods for the Examination of Foods were used [19]. Samples were tested for total aerobic counts and coliforms. One mL of the above untreated grains suspension was pour-plated on plate count Agar (Oxoid CM 463 / 325), and on violet red bile agar (Difco lab, Detroit, MI) then incubated for 48 h at 30 and 37°C, respectively. Duplicate agar plates of between 30 and 300 colonies were counted, and mean counts calculated. The counts for each plate were expressed as the \log_{10} of the colony forming unit of the suspension (cfu/g).

2.3 Statistical Analysis of Data

All values were expressed as the mean of three measurements for each experiment. Mean values of proximate composition were compared for the different cultivars. Relating to microbial experiment growth, mean bacterial and fungal counts were compared for the different cultivars. The data analysis involved a two-way analysis of variance (ANOVA). Mean differences were determined using a “*t*-test”. A significant difference was established ($P < .05$).

3. RESULTS AND DISCUSSION

The composition of the cowpea flour from the two cultivars tested is summarized in Table 1. The chemical analysis of the seeds revealed that the major compounds were carbohydrate and proteins with slightly higher values for the RC (48.35 % and 28 % for RC, 47.99 % and 26.25 % for WC). Protein values found in this study are also in agreement with previous reports [3,20,21]. According to Shimelis et al. [22], protein contents in legume grains range from 17 to 40% similar to the protein content of meat (18 - 25%) but higher than the 7 to 13% found in cereal grains.

In general, the protein content was higher in the RC. Tharanathan and Mahadevamma [23] demonstrated that the protein in cowpea seeds contains more amino acids, lysine and tryptophan, compared to cereal grains; however, it is deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seeds are valued as a nutritional supplement to cereals and an extender of animal proteins.

The starch content was 41.66% and 40.05% for RC and WC. The carbohydrate and starch contents in this study were similar to what was reported by Souci et al. [24] showing 46.93 % for carbohydrate and 44.82 % for starch. The white cultivar showed a significantly ($P < 0.05$) higher total sugar content compared to the Red cultivar. The lipid content (2%) in both cultivars studied was below the one reported by Nwaga et al. [25] for the IT81D-985 cultivar of cowpea with 6 %, but close to the values (1.58%) reported by Souci et al. [24]. The low lipid content of the cultivars studied suggests that cowpeas should be consumed together with high lipid content foods.

The ash contents (Table 1) for both cultivars (3.54 and 3.79 %) were not significantly different ($P > .05$). The results obtained agree with those reported by Abusin et al. [26] who found a value of 3.47% for faba bean cultivars. The Ca, P and Mg values of 54.72; 430.01; 119.22 mg/100g for RC and 48.67; 429.03; 131.70 mg/100g for WC were lower than the values reported by Souci et al. [24], (107.83; 460.59 and 281.53 mg/100g dry matter). The iron content (15.95 -13.91mg/100g) would suffice the daily requirement for human which is between 5 and 10 mg/100g/day [27]. Legume grains are also an important source of minerals (Ca Fe, Cu, Zn, P, K, Mg), and contain very low sodium. The mineral content recorded (calcium, phosphorous, copper, iron and zinc) were not significantly different ($P > .05$) for both cultivars except for the magnesium which was higher ($P < .05$) for the white cultivar. Ca, P and Mg were below the values reported by Souci et al. [24]. This reported mineral content can cover the daily requirement for human which is between 5 and 10 mg/day [27].

Table 1. Effect of water-soaking on the chemical composition of Red and White cultivars of cowpea (*Vigna unguiculata*) dried grains

Cowpea dried grains				
Composition (g/100g dwb)	Red Cultivar		White Cultivar	
	Non soaked	Water-soaked	Non soaked	Water+soaked
Moisture	8.76 ± 0.69*	22.00 ± 1.83*	9.07 ± 0.87*	39.26 ± 1.87*
Total carbohydrate	48.35 ± 2.97*	34.70 ± 1.95*	47.99 ± 0.68*	33.93 ± 2.50*
Total Sugar	2.07 ± 0.02	2.06 ± 0.01	3.49 ± 0.00*	2.31 ± 0.01*
Starch	41.66 ± 2.65*	29.38 ± 1.77*	40.05 ± 0.59*	28.46 ± 2.24*
Lipids	2.52 ± 0.35	2.13 ± 0.00	2.44 ± 0.13	2.08 ± 0.12
Proteins	28.00 ± 0.16*	27.12 ± 0.11*	26.25 ± 0.20*	23.62 ± 0.08*
Cellulose	6.00 ± 0.20	5.33 ± 0.14	3.67 ± 0.09*	3.00 ± 0.14*
Ash	3.54 ± 0.11	3.45 ± 0.09	3.79 ± 0.04*	3.32 ± 0.13*
pH	6.64 ± 0.03	6.67 ± 0.04	6.66 ± 0.03*	6.92 ± 0.03*
acidity (meq/100g)	10.58 ± 1.86	9.59 ± 0.33	10.03 ± 1.22*	7.53 ± 0.07*
Energy (cal/100g)	328.13 ± 2.67*	266.45 ± 7.35	315.94 ± 2.79*	246.38 ± 11.98*

Means with * in the same line are significantly different ($P < .05$); Means without* are not significantly different ($P > .05$). The differences within means should be read for each cultivar.

The moisture content for both cultivars was relatively low (8.76 to 9.07%). This result could explain the long storage stability of these dried legume products that can reach up to 4 months as the relatively low moisture content of the peas inhibits a microbial growth. Both cultivars have a high energy value (315.94 to 328.13 cal/100g of seeds), that suggests that they can meet the daily energy requirement of the populations. In general, legumes are sources of dietary fibres, having a significant amount of vitamins and minerals, and a high energy value [23]. However, the preparation processes (soaking, cooking...) could cause nutrient losses and affect the energy value of these products. Titratable acidity of both

cultivars was low while the pH was slightly acidic. But the storage conditions may favour a microbial growth especially of mould which under high relative humidity conditions could produce mycotoxins in the grains.

A decrease of the major components from water-soaked grains was observed (Tables 2-3). Water soaking resulted in a mean reduction of 3.14% and 10.02% protein, 28.23% and 29.30% carbohydrate, 29.47% and 28.94% starch, 2.54% and 12.40% ash, 18.80 and 22.02% energy for Red and White cultivars respectively. A decrease of mineral content was also observed (Table 4). Water soaking resulted in a mean decrease of 23.13% and 47.66% iron, 2.32% and 8.15% calcium, 9.30% and 2.10% phosphorus for RC and WC respectively. The loss of nutrient content for total sugar was 33.81% followed by carbohydrate (29.30), starch (28.94%), energy (22.02%) and cellulose (18.25%) for WC. For the RC, there was almost no sugar loss (0.48 % vs. 33.81% for WC). There were some losses of protein (3.14 %) and ash (2.54%), but less when compared to the losses from the WC (10.02% and (12.40%) after water soaking.

Table 2. Effect of water-soaking on the mineral content of red and white cultivar of cowpea (*Vigna unguiculata*) dried grains

Mineral (mg/100 g)	Cowpea dried grains			
	Red cultivar		White cultivar	
	Non soaked	Water-soaked	Non soaked	Water-soaked
Calcium	54.72 ± 0.00	53.45 ± 1.26	48.67 ± 1.63	44.70 ± 0.48
Phosphorus	430.01 ± 0.00	390.00 ± 0.01*	429.03 ± 0.11*	420.00 ± 0.09*
Copper	0.72 ± 0.00	0.71 ± 0.00	0.77 ± 0.00	0.68 ± 0.00
Zinc	3.99 ± 0.00	3.55 ± 0.00	3.86 ± 0.00	3.73 ± 0.00
Magnesium	119.22 ± 1.54	120.05 ± 0.05	131.70 ± 3.28	112.09 ± 0.00
Iron	15.95 ± 1.03	12.26 ± 0.76	13.91 ± 1.63*	7.28 ± 0.72*

Means with * in the same line are significantly different ($P < .05$); Means without* are not significantly different ($P > .05$). The differences within means should be read for each cultivar.

Table 3. Incidence of water-soaking on the percentage (%) of components loss for the Red and White cultivars of cowpea dried grains

Cowpea cultivars	Moisture	Total carb	Total Sugar	Starch	Lipids	Proteins	Cellulose	Ash	Energy
Red	251.14	28.23	0.48	29.47	15.47	3.14	11.16	2.54	18.80
White	333.33	29.30	33.81	28.94	14.75	10.02	18.25	12.40	22.02

During the soaking process, hydrated grains tend to lose some of the chemical and nutritional components as evidenced by a decrease in chemical components of the soaked grains. There were also some differences between the RC and the WC as shown in Table 4: ash (2.54% for RC vs 12.40% for WC), iron (23.13 for RC vs 47.66% for WC) and titratable acidity for the WC. Results indicate that the mineral leaching was more for the WC than the RC. Subsequently the soaking process affects more the composition of the WC than RC. The difference in nutritional component loss between WC and RC can be explained by shell and the internal structure of the grains [28]. WC has a fine, hard and rough shell compared to RC with a smooth surface shell, loosely adhering seed coat, without any space in the internal structure, causing difficulty in the adhesion of water molecules to the surface (hydrophobic), resulting in less water absorption. Yousif et al. [29] have recently shown that empty spaces in the internal structure of these grains facilitate permeability and components

solubility and thus, leading to a removal of nutrient from the internal structures. According to Mayer & Podjakoff-Mayber [27], the main component which absorbs water in seeds is the protein. Since the structure of RC membrane seems less hydroscopic, less leaching of nutrients occurred from the internal structures.

Table 4. Incidence of water-soaking on the percentage (%) of mineral content loss for the Red and White cultivar of cowpea dried grains

Cowpea cultivars	Calcium	Phosphorus	Copper	Zinc	Magnesium	Iron
Red	2.32	9.30	1.39	11.02	-	23.13
White	8.15	2.10	11.69	3.36	14.89	47.66

The microbiological count is given in Fig. 1. Mean count (Log_{10} cfu/g) of total aerobic microflora, coliforms, mould and yeast of 6.43 and 6.3; 2.58 and 2.04; 4.8 and 4.39 were obtained respectively for WC and RC. There were no significant differences ($P < .05$) between WC and RC for total aerobic microflora, coliforms, mould and yeast. These results were in accordance with the work done by Bulgarelli et al. [30] on the microbiological quality of cowpea paste. These authors indicated that the predominant bacteria consisted of *Enterobacter*, *Klebsiella*, and *Lactobacillus* species. The low water activity (0.70) of the grains can't allow the growth of most microorganisms. Grains are known to be contaminated by several microorganisms such as bacteria and fungi. Since the grains were stored in open dishes and manipulated by the sellers in non hygienic conditions, coliforms can contaminate the grains. The mycoflora was mainly represented by five genera: *Aspergillus*, *Mucor*, *Penicillium*, *Botrytis* and *Geotrichum*. The predominant fungi belonged to *Aspergillus* genus. The total aerobic microbial loads were generally higher in the white grain than in the Red grain samples. The less bacteria counts in the RC grains and the genus of mould isolated were probably due to the fact that RC grains have a smooth surface shell, not easily permeable to most micro organisms. The isolated species were similar for both cultivars demonstrating that the contamination came mainly from the air rather than from the grains.

Aspergillus strains were isolated from all (approximately 100%) the samples analysed in either treated or untreated grains (Figs. 2-3), followed by *Mucor* and *Penicillium* (100% and 2% for WC and RC). *Geotrichum* and *Botrytis* were not found in the RC but *Botrytis* was found only in the WC. Treated RC seems to be less contaminated. WC was contaminated with the 5 genera of mould. These results agreed with those of Hitokoto et al. [5] who found that genera *Penicillium* and *Aspergillus* were the major contaminants of beans. These results were supported by Bulgarelli et al. [30] and also by Ihejirika [31] who recorded the presence of *Aspergillus niger* as the predominant species and also a high occurrence of fungal diseases in cowpeas. The presence of *Aspergillus* implies a risk of aflatoxin production and could represent a health risk for the consumers. The WC seems more contaminated by fungal than the RC. Differences in the percentage of fungal isolates have been detected among all the cowpea grain samples analysed (Figs. 2-3). A high percentage of mould could be favoured by high moisture content. In this study, the moisture contents were 8.76% and 9.07% for RC and WC, indicating that the grains could be susceptible to a mould growth.

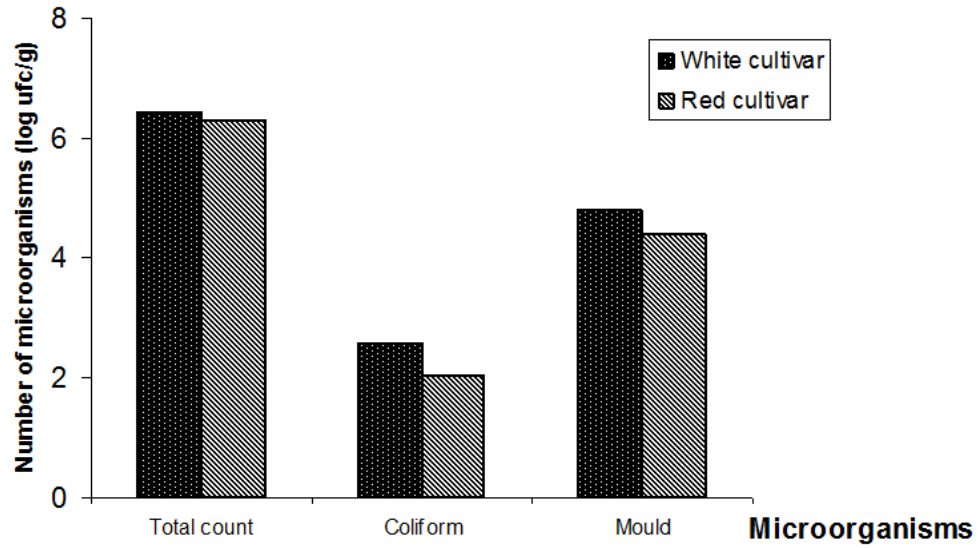


Fig. 1. Mean values of microbiological count in cowpea dried grain cultivars

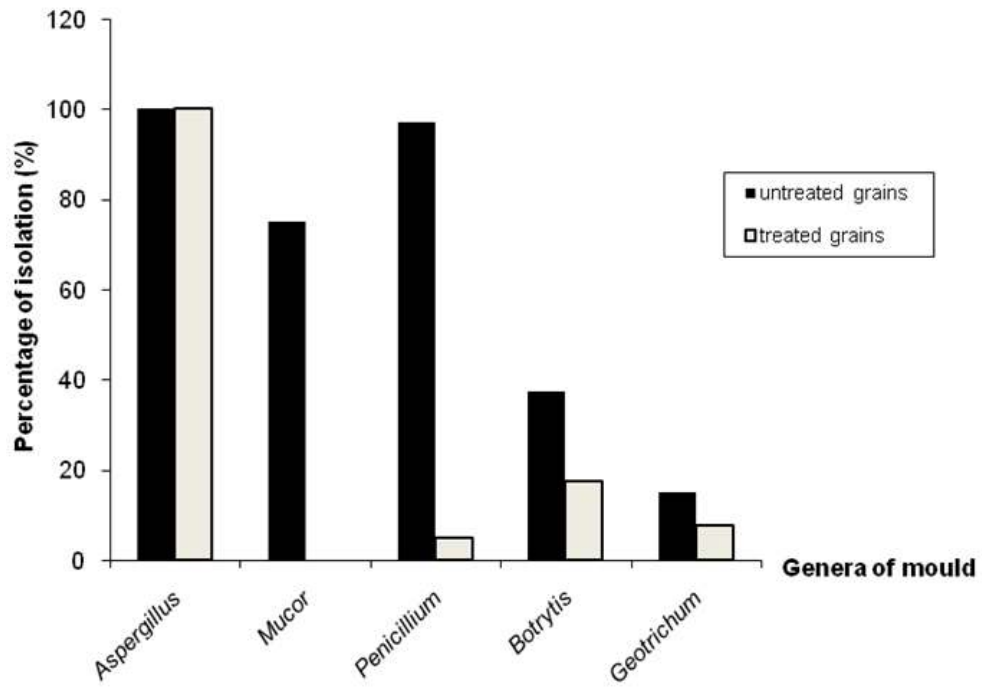


Fig. 2. Frequency of isolation of mould in the white cowpea grains cultivar

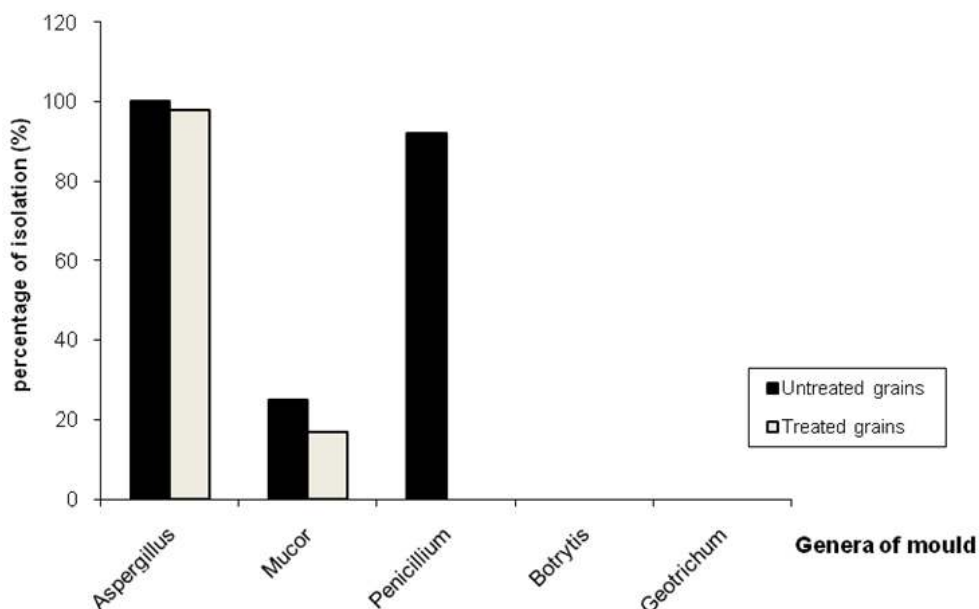


Fig. 3. Frequence of isolation of mould in the red cowpea grains cultivar

In Côte d'Ivoire, cowpea grains are stored and sold at high relative humidity and ambient temperature. These conditions can favour the growth of fungi since a high ambient temperature increases a microbial growth. Then, the microbes can cause spoilage of the cowpeas, soften the tissue and encourage a further microbial growth. Diseases infestations lower the seed viability [32]. According to Magnoli et al. [33], fungal contaminants are responsible for a substantial damage in stored food products including discolouration, loss in nutritional value, production of off-odours, deterioration of the product quality and production of mycotoxins.

In all the countries with a warm and wet climate, the moisture and poor agronomic conditions are favourable to a fungi growth and product contamination which could consequently affect the food quality. Food prepared with defective grains could produce an unpleasant flavor that is unpalatable to human consumption. Poor quality cowpeas impact the marketability of the product. Tournas and Katsoudas [34] assumed that fungal spoilage of crops will depend on the cultivar, the cultivation and harvesting techniques, handling, transport, and post-harvest storage and marketing conditions.

4. CONCLUSION

Water soaking may affect the content of bioavailability of cowpea nutrients. During the water soaking, the grains lose some nutrient contents such as protein and carbohydrate. Cowpeas represent an ideal nutritious alternative crop, especially in Côte d'Ivoire and other developing countries, where extensive food production should be cost effective, with minimal input from growers. The cowpea grains are an inexpensive source of protein in the diet. However, the cultivar types and the preparation methods could affect the nutrient availability. The white cultivar may be best utilized for industrial processes where gel formation is not required.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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