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Nutritional Properties and Biochemical Effects of *Pachira Insignia* Cakes Treated by Grilling and Cooking in Young Wistar Rats

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Abstract: *Pachira insignis* seed cakes, treated raw, roasted, or boiled, in young male Wistar rats. The cakes were characterized physicochemically , showing protein contents of 8.37 to 11.03% and variations in total sugars, phenolic compounds, and tannins depending on the treatment. Twenty-five rats were divided into five groups, including a negative control, a casein-positive control, and three groups fed the respective cakes as a protein source. The 13-day experiment revealed an initial weight gain in the rats fed the roasted and boiled cakes, followed by a decline, while the raw cake group did not survive. Biochemical analyses showed signs of toxicity, including elevated levels of urea, creatinine, and transaminases (ALAT and ASAT) in the treated groups compared to the casein group. The organ masses (heart, liver, kidney, testes) of rats in the grilled and boiled groups were greater than those in the casein group. These results indicate that *Pachira* cakes *insignis,* regardless of the preparation, exhibit toxicity in young rats, which limits their use as a protein source.
Keyword: *Pachira insignis*, oilcakes, physicochemical characterization, proteins, nutritional properties

INTRODUCTION

Malnutrition remains a major challenge in sub-Saharan Africa, where despite continued efforts, the situation remains worrying (UNICEF, 2021). In 2018, approximately 239 million people (22.8% of the population) were undernourished in the region, with an increase in prevalence since 2014, particularly in West and Central Africa. Among children under five, 32.6% suffered from stunting in 2017, while 6.9% were acutely malnourished. To address this, it is crucial to explore protein-rich plant-based food resources, including legumes, known for their high protein and essential mineral content (Cheftel & Cheftel, 1990; Young & Pellet, 1994). However, these seeds often contain anti-nutrients such as tannins, phytates and trypsin inhibitors, which reduce protein digestibility and nutrient bioavailability (Jenkins, 1994; Smith *et al.*, 2020).

Among legumes, soybeans and peanuts are major protein sources benefiting from improved production worldwide (FAO, 2022). Meals from these seeds represent an important source of protein, particularly in animal feed, the demand for which is growing in parallel with that of animal protein (Dronne, 2001; Wang *et al.*, 2019).

Some underexploited legumes, such as *Pachira insignis* (Malvaceae family), remain unknown to the general public. The seeds of *P. insignis* are traditionally used in human food, particularly to thicken sauces or eaten as snacks (raw, roasted or boiled). Analyses have revealed that these seeds contain approximately 50% lipids and 16% proteins (Abini , 2012), with a high glutamic acid content (20.4%) (Ata, 1973). They also contain antinutrients such as 3.5% tannins, 7.41 mg/g phytates, 3.87 mg/g oxalates and 2.11% alkaloids (Ibiyinka *et al.*, 2011). The extracted oils present toxicity linked to the presence of cyclopropenoic fatty acids (Yeboah *et al.*, 2011).

Few recent studies have evaluated the impact of these components on growth and metabolism,

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particularly with regard to the nutritional quality of proteins contained in P. *insignis meal*. In vivo digestibility assessment remains an essential method in nutritional physiology to assess protein quality, integrating mechanical, chemical and enzymatic factors (Adrian *et al.*, 1998; Mezajoug, 2010; Lopez *et al.*, 2023). Furthermore, various preliminary treatments such as soaking, cooking, germination, fermentation, grilling or roasting are recognized to improve the nutritional quality of feed by reducing antinutrients (Richard *et al.*, 1997; Ngatchic *et al.*, 2013; Kumar *et al.*, 2021).

Given the significant levels of antinutrients and potentially toxic substances in *P. insignis* seeds , the central question of this study is: what is the influence of thermal treatments (cooking and roasting) on the physicochemical composition of *P. insignis cakes* and on the in vivo digestibility of their proteins? hence The objective of this study is to evaluate the effect of heat treatments (cooking and roasting) on the nutritional properties of ***P. insignis cakes*** and their impact on growth and biochemical parameters in young albino rats.

MATERIALS AND METHODS

Type, Framework and Period of Study

Type and Period of Study

Study Framework

This work was carried out at the Laboratory of Biophysics, Food Biochemistry and Nutrition (LABBAN) of the National School of Agro-Industrial Sciences (ENSAI) and the Biology Laboratory of the Faculty of Sciences of the University of Ngaoundere Material

Plant material

The plant material used consists of mature *Pachira insignia* seeds collected in March 2015 in the locality of *Idool*, which is a village located 70 km east of Ngaounderé, or 120 km via Tello, and transported to the Biophysics, Food Biochemistry and Nutrition (LABBAN) laboratory of ENSAI for studies.

Animal material

The animal material consists of young male rats of the Wistar strain aged 3 to 4 weeks weighing between 39-59g from the animal facility of the National School of Agro-Industrial Sciences (ENSAI) where the experiments took place. The choice of this strain is justified by its sensitivity to environmental and infectious factors; the most obvious advantage is its omnivorous character and especially the sensitivity of its response to nutritional conditions (Adrian *et al.,* 1998).

METHODS

Pachira Insignia Cake

Once in the laboratory, the seeds were trilled and divided into 3 batches. The 1st batch was roasted at 170 °C for 30 minutes in a roaster (figure 5a), the 2nd was cooked at 100 ° for 50 minutes in a water bath (figure 5b) and the 3rd did not undergo any treatment. After cooling, the roasted seeds were directly ground using a manual machine (Victoria) and as for the raw and boiled seeds, they were dried at room temperature for 48 hours and subsequently ground with the same grinder. The pastes obtained were used for the production of different cakes by hexane delipidation using the soxhlet . Thus, a mass of the different pastes was bagged in filter paper and introduced into the soxhlet containing 1 liter of hexane in its flask. And the oil from these flours was extracted for 8 hours of time, then the cakes were recovered (fig1).

Figure (1) shows the production diagram of *P. insignis oilcake*

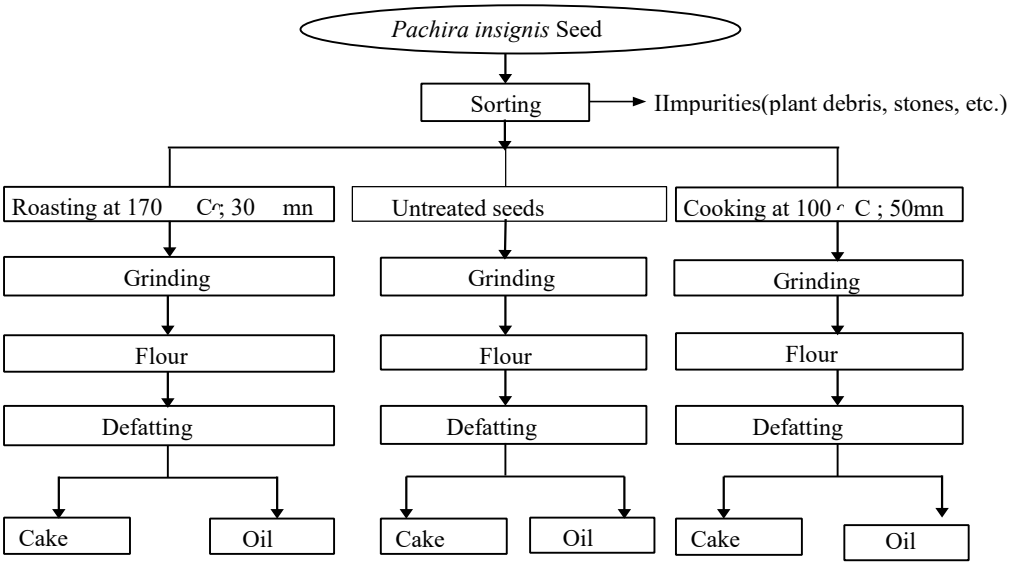

Figure 1: *Pachira insignia* oilcake production diagram

Determination of the content of some nutrients

Water content and dry matter

The water and dry matter content were determined according to the AFNOR method (1981). For this, a fresh sample of approximately 3 g was dried at 105 °C for 24 hours in an oven until constant weight. The dry matter corresponds to the total dry residue, expressed as a percentage of the fresh matter, while the water content is calculated by difference.

Lipid content

Total lipid content was measured by Soxhlet extraction with hexane for 8 hours. 50 g samples were placed in filter paper bags and then extracted. The amount of oil was calculated from the difference in weight of the bags before and after extraction, expressing the oil content per 100 g of dry sample.

Protein content

Soluble protein determination was performed using the Folin -Lowry colorimetric method, based on the formation of a blue complex measured at 650 nm. Reagents were prepared according to a specific protocol, and sample absorbances were compared to a calibration curve obtained with bovine serum albumin solutions.

Total sugar content

The total sugar content was determined by the phenol-sulfuric acid method (Dubois *et al.*, 1956), where the sugars undergo a reaction in a hot acid medium, forming a yellow-orange colored complex measured at 490 nm. Glucose was used as a standard to establish the calibration curve.

Reducing sugar content

Reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNS) method (Fischer & Stein, 1961). In alkaline and heated media, DNS reacts with reducing sugars, producing an orange complex measured at 540 nm. Extraction was by heating with boiling water, followed by filtration and colorimetric determination.

Determination of total phenolic compounds

For the determination of total phenolic compounds, the extracts were prepared with 70% ethanol and then assayed with the Folin-Ciocalteu reagent. The reaction produces a blue color measured at 725 nm, proportional to the phenol concentration, with gallic acid as the standard.

Tannin dosage

Tannin content was assessed using a modified method of Makkar (2003), using polyvinylpolyrrolidone (PVPP) to bind tannins and separate them from other phenols. Tannin content is obtained by the difference between the total phenol content and non-tannic phenols.

This methodology allows for a complete characterization of the main nutrients and antinutrients present in the samples analyzed.

Effect of oilcake consumption (grilled, boiled and raw) on growth and some biochemical parameters of rats.

Formulation of diets

This study focuses on the protein quality of *Pachira cakes insignis* from raw, cooked, and roasted seeds. Diets were prepared in the laboratory according to the method of Bouafou *et al.* (2007), with some adaptations. Five separate diets were developed for each group of animals: a negative control group with no protein source, a positive control group using casein as a protein source, and three experimental groups receiving roasted, boiled, or raw oilcakes, each supplemented with 5% casein (see Table 1).

Table 1: Composition of rat food rations g/100g of food

	Diets				
Ingredients	Protein-free diet (PFD)	Casein	TG	TB	TC
Multivitamins	1	1	0.43	0.43	0.43
Bone powder	4	4	1.7	1.7	1.7
Cellulose	5	5	2.13	2.13	2.13
Sunflower oil	10	10	10	10	10
Casein	-	10	5	5	5
Grilled crab	-	-	42.5	-	-
Boiled crab	-	-	-	42.5	-
Raw crab	-	-	-	-	42.5
Cassava starch	80	70	38.25	38.25	38.25
Totals (%)	100	100	100	100	100

TG: Grilled crab, TB: Boiled crab, TC: Raw crab, RSP: Protein-free diet

Animal experimentation

After an acclimation period of [duration to be specified], 25 albino rats were divided into experimental groups. The experiment lasted approximately two weeks. Each morning, between 8:00 and 10:00 a.m., food, prepared in the form of balls, was weighed and then distributed to the animals. Water was provided ad libitum in bottles, renewed every other day. Before each distribution, food leftovers were collected and weighed to calculate the actual consumption of each group

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(Adrian *et al.*, 1998). The animals were weighed on the first day, then every other day at the same time, with a final weighing at the end of the study.

Sacrifice and biological samples

At the end of the experiment, the rats were euthanized under petroleum ether inhalation anesthesia. Blood was collected by incision of the jugular vein and then centrifuged at 3000 rpm for 15 minutes to isolate the serum, which was stored at -20 °C for biochemical analyses. Organs (liver, kidneys, heart, testes) were removed, preserved in saline solution (NaCl 0.9%) and weighed for the determination of organ indices.

Organ biometrics

The organ index, expressing the relative weight of each organ compared to body weight, was calculated according to the following formula:

Organ index (%) = organ mass (g) / body mass (g) ×100

In vivo nutritional value assessment

Pachira cakes *insignis* was assessed from the growth parameters and nitrogen balance of rats, according to the standard methods recommended by Jood & Singh (2001) and Pellet & Young (1980). The main parameters measured were:

- **Dry matter ingested (DMI)**: total quantity of dry matter consumed during the experimental period.
- **Weight gain (WG)**: difference between the final weight and the initial weight of the animals.

Assay of blood biochemical parameters in young rats

Aminotransferase activities (AST and ALAT) were determined by the colorimetric method of Reitman and Frankel (1957), based on the formation of 2,4-dinitrophenylhydrazone derivatives measured at 505 nm. Urea was measured by enzymatic hydrolysis followed by a coupled reaction with glutamate dehydrogenase, with spectrophotometric reading at 600 nm. Creatinine was quantified according to the Jaffé method, by measuring the intensity of the color of the creatinine-picric acid complex at 505 nm. All assays were performed on serum obtained after centrifugation of blood samples.

Statistical analyses

Results are presented as means ± standard deviation or percentage. Data were analyzed by analysis of variance (ANOVA), followed by Duncan's multiple comparisons test to identify significant differences between groups. Statistical analyses were performed using Statgraphics Plus 5.0 software (Manugistics , Rockville, Maryland, USA, 1997). Graphs were generated with Sigmaplot 11.0 software (Systat Software, California, USA). A threshold for statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physicochemical composition of *Pachira seed cakes* The results of the analyzed physicochemical parameters are presented in Table 2**.**

Table 2: Nutrient and antinutrient content of *P. insignis* seed cake (g/100 g dry matter)

	PG	PB	PC
Dry matter	97.76±1.39 ^{c}	58.82±0.16 ^{a}	94.20±0.10 ^{b}
Water	1.70±0.46 ^{a}	41.12±0.10 ^{c}	5.76±0.09 ^{b}
Total protein	8.37±0.28 ^{a}	11.03±0.80 ^{c}	10.06±0.37 ^{b}
Phenolic compounds	0.12±0.07 ^{a}	0.09±0.05 ^{a}	0.19±0.05 ^{a}
Tannins	0.059±0.01 ^{b}	0.036±0.01 ^{a}	0.075±0.01 ^{c}
Reducing sugars	19.78±2.17 ^{b}	47.61±13.18 ^{c}	16.08±1.14 ^{a}
Available sugars	21.02±3.92 ^{c}	12.31±3.61 ^{b}	6.85±0.29 ^{a}
Total reducing power	3.17±0.35 ^{b}	1.88±0.43 ^{a}	3.24±0.16 ^{c}

PG = Pachira grilled; PB= Pachira boiled; PC = raw Pachira . Means ± standard deviation followed by the same letter in superscript on the same line are not significantly different at the P< 0.05 threshold

Table 2 shows the chemical composition of *cakes Pachira insignis* subjected to different treatments (roasting, cooking and raw). A very significant variation ($P < 0.001$) was observed in the dry matter and water content between the three types of cakes. The water content is lowest in the roasted cake (1.70 ± 0.46%), intermediate in the raw cake (5.76 ± 0.09%) and highest in the boiled cake (41.12 ± 5.76%). these results obtained on the physicochemical composition of *cakes Pachira insignis* reveal significant variations induced by thermal treatments, particularly roasting and boiling. The significant decrease in water content during roasting is explained by the rapid dehydration of the seeds, promoting the concentration of nutrients and the activation of Maillard reactions, responsible for the observed brown coloration (Baltes & Bochann , 1987). This phenomenon is common in dry thermal processes and has also been reported in other vegetable cakes, where moisture loss modifies the texture and stability of the product (Ntsoumou *et al.*, 2023). The increase in water content during boiling is related to seed imbibition, a process that modifies the cellular structure and facilitates the release of certain soluble compounds. This increased hydration may influence nutrient digestibility and availability, as shown by several studies on similar cakes (Ibiyinka *et al.*, 2011; Bernadete *et al.*, 2010).

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Concerning The protein content decreases in the roasted cake (8.37%) compared to the raw cake, while it increases in the boiled cake (11.03%). The reducing sugar content also varies according to the treatment, ranging from 16.08% in the raw cake to 47.61% in the boiled cake, with an intermediate value of 19.78% in the roasted cake. Therefore, the protein content, the decrease observed in the roasted cake is attributable to thermal denaturation and the participation of proteins in complex reactions such as Maillard reactions, which can reduce the availability of essential amino acids (Gaulier & Serres, 1971). Conversely, cooking in water seems to favor the release of structural proteins, probably by breaking cellular bonds and limiting polymerization reactions, which increases the measured protein content. These observations corroborate the results obtained on other cakes, where the treatment method strongly influences the protein quality (Ntsoumou *et al.*, 2023).

For available sugars, the contents are respectively 21.02 ± 3.92% (roasted), 12.31 ± 3.61% (boiled) and 6.85 ± 0.29% (raw). Total phenolic compounds do not show any significant difference between treatments (0.12 ± 0.07% for roasted, 0.09 ± 0.05% for boiled and 0.19 ± 0.05% for raw). On the other hand, the tannin content decreases significantly in roasted (0.059%) and boiled (0.036%) cakes compared to raw (0.075%). The significant increase in reducing sugars in boiled cakes can be explained by the partial degradation of complex polysaccharides into simple sugars, as well as by the weakening of cell membranes facilitating their extraction. This trend is also observed in other seeds and cakes subjected to wet heat treatments (Abini , 2012).

Total phenolic compounds, on the other hand, appear to be little affected by the treatments, suggesting a certain stability of these antioxidants under moderate roasting or cooking conditions. However, the significant reduction in tannins, particularly in boiled cake, is consistent with their water-soluble nature and their sensitivity to aqueous treatments. This reduction may have a beneficial effect on the digestibility and nutritional value of cakes, as tannins are known for their ability to complex proteins and reduce their absorption (Ibiyinka *et al.*, 2011).

Finally, the comparison with other vegetable cakes, such as those from cotton, peanut or baobab, shows that the chemical composition of *Pachira cakes insignis* is comparable in terms of proteins and lipids, but that variations linked to heat treatments are a determining factor in their nutritional quality (Gaulier & Serres, 1971; Lescoproduits , 2016). These results highlight the importance of controlling processing methods to optimize the nutritional value of oilcakes intended for animal or human food.

***Pachira insignia* cakes and proteins**

Food consumption and nutrient ingestion

Figure 2 shows the quantity (in g) of food ingested.

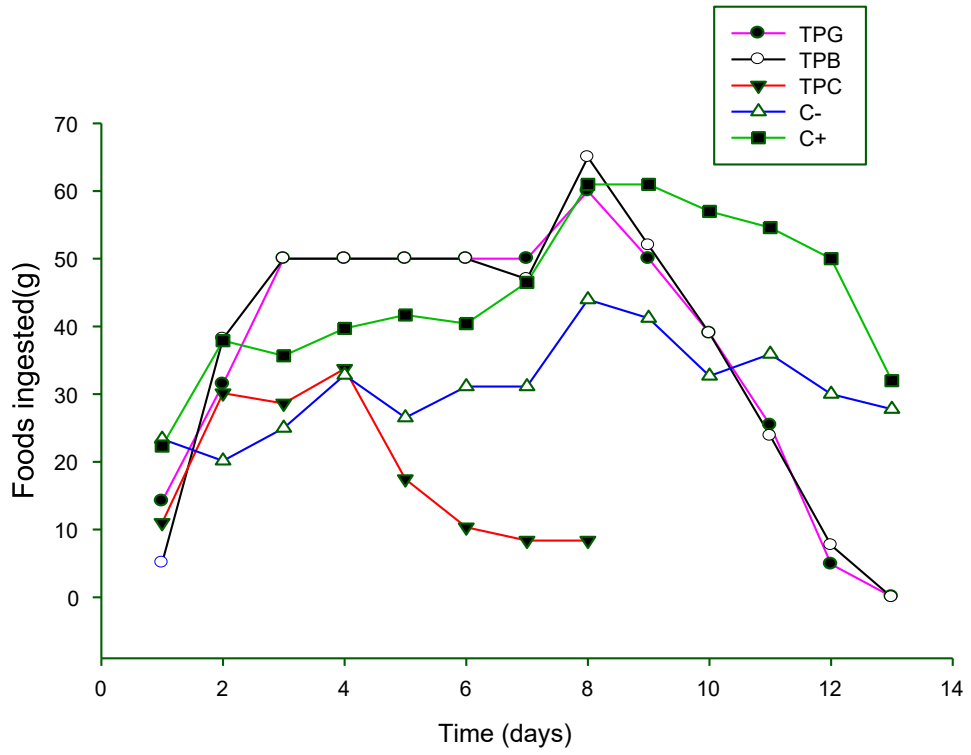

Figure 2: Effects of feeding on young rats

TPG = Pachira Crab grilled; TPB = Pachira crab boiled; TPC = Pachira Crab raw; C- Negative control; C+ Positive control

The curves show that the group receiving casein (C+) has the highest food consumption, with an average of 42.68 g, while the group without protein intake (C-) has the lowest consumption, 29.89 g. The groups fed with *Pachira oilcakes The grilled (TBG) and boiled (TPB) insignis groups* showed similar profiles, with peak consumption around 60 and 65 g respectively, and average consumption of 37.21 g and 37.29 g. The group receiving the raw crab (TPC) did not survive the experiment.

The observed difference in feed intake between groups is likely related to the treatments applied to the seeds. The high palatability of the casein-based diet explains the high consumption in this group, while the total absence of protein in the C- group reduces feed quality and therefore its consumption. The peaks in consumption of the roasted and boiled oilcakes could be due to the organoleptic changes induced by these treatments.

However, these cakes appear to be toxic to young rats. Indeed, the TPC group, which received the raw, untreated seeds, began refusing food as early as day 4, likely due to the antinutrients and toxicity that manifested, leading to mortality. The TBG and TPB groups initially showed exponential consumption, suggesting that the treatments enhanced the attractiveness of the seeds. After day 3, consumption stabilized until day 7, then dropped sharply to less than 1 g per day, leading to the animals being sacrificed on day 3.

Evolution of weight gain (g every 2 days) during the experiment

Figure 3 shows the weight gain acquired during the experiment.

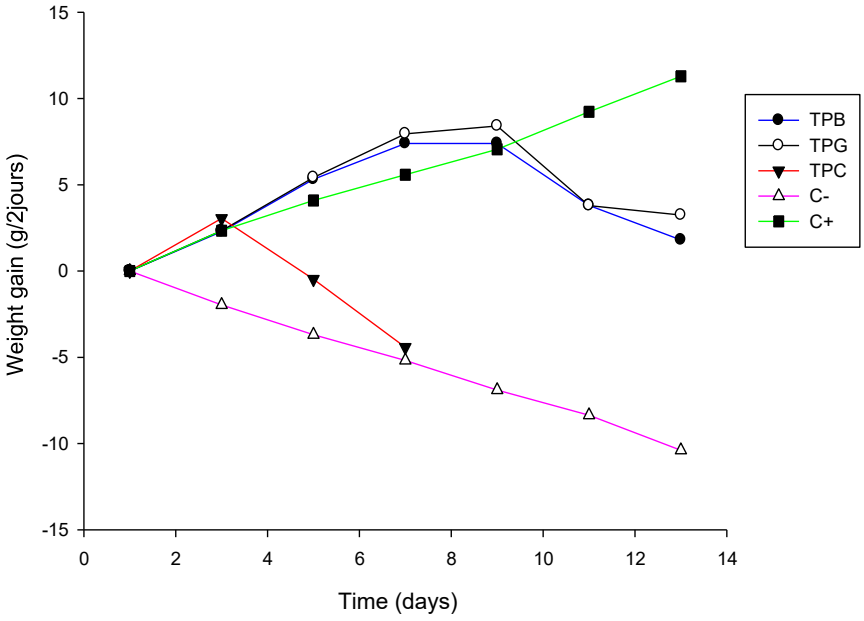

Figure 3: Evolution of the weight of rats according to the different protein diets

TPC = Pachira Crab raw; TPB = Pachira crab boiled; TPG = Pachira Crab grilled; C+ = Positive control; C- = Negative control.

Figure 3 illustrates the evolution of the rats' weight as a function of time and diet received. A weight gain of 11.28 g was observed in the casein-fed group (group C+) on day 13, while the group without protein intake (group C-) suffered a weight loss of -10.38 g. This difference is explained by the superior quality of casein, a reference protein containing the eight essential amino acids necessary for growth, unlike the negative control group which received no protein source, leading to a deterioration in its body condition.

Pachira cakes *Grilled (TPG) and boiled (TPB) insignis* show similar weight gains, peaking at 7.38 g and 8.40 g respectively on day 9, before dropping to 1.8 g and 3.2 g on day 13. This decline suggests that from day 8 onwards, feed quality deteriorates, leading to reduced animal consumption.

The group fed with raw meal (TPC) began to lose weight as early as day 3, with mortality occurring on day 7, indicating significant toxicity related to the absence of treatment. Despite this, the TPG and TPB curve profiles suggest that these meals could be an

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interesting protein source if their toxicity were controlled.

These results differ from those reported by Ngatchic *et al.* (2013b) and Mezajouk (2010), who observed a significant increase in body mass in rats fed fermented soy milk, highlighting the variable impact depending on the protein source and treatment.

Some blood biochemical parameters

a) Creatinine

Creatinine is a breakdown product of creatine metabolism, primarily from muscle. Blood creatinine measurement, called serum creatinine, is a reliable indicator of kidney function. Normal blood creatinine levels reflect adequate kidney function, while elevated blood creatinine levels are generally associated with kidney failure (Paillard, 1987).

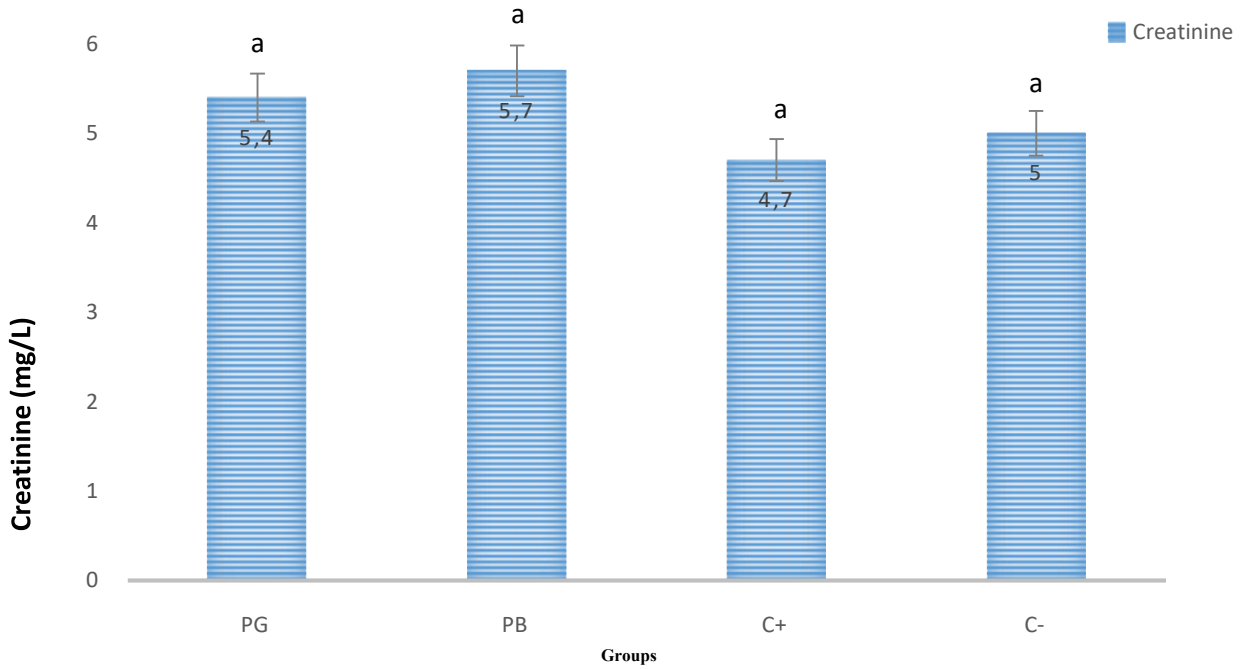

Figure 4: Influence of different diets on serum creatinine

Serum creatinine concentrations measured in rats fed with *Pachira meal Insignis* roasted (5.4 mg/ dL) and boiled (5.7 mg/ dL) did not differ significantly ($P < 0.05$) from those observed in the casein-fed control group (5.0 ± 1.69 mg/ dL). This similarity indicates that the treated cakes did not cause significant alteration of renal function in the animals during the experimental period.

Blood creatinine is a recognized marker of renal function, reflecting glomerular filtration (Boisvert, 2009; JoVE , 2024). Normal levels suggest efficient glomerular filtration and an absence of renal failure. Thus, the absence of serum creatinine elevation in the TPG and TPB groups indicates that these protein diets do not compromise the renal health of rats, despite the potential presence of antinutritional compounds in the meal.

Previous studies on other animal species confirm that moderate variations in serum creatinine are compatible with normal renal function, particularly in rabbits fed with local food resources (Atchade *et al.*, 2019 or during phytotherapeutic treatments (Yapo Adou *et al.*, 2014). In addition, the assessment of renal function by classical biochemical markers remains a reliable method in nutritional toxicology (Bello *et al.*, 2016; JoVE , 2025). These results suggest that *Pachira* cakes *Grilled or boiled insignis* can be considered as protein sources without apparent adverse effects on renal function in the short term. However, longer-term follow-up and additional analyses (renal clearance, histopathology) would be necessary to confirm this tolerance.

b) Urea

Urea is the main end product of amino acid breakdown in the body. It is synthesized in the liver via the urea cycle, or ureogenesis, a key metabolic process that converts toxic ammonia into a less harmful molecule (Walker & Harper, 2014). Once formed, urea is transported into the blood and then eliminated primarily by the kidneys through urine, with an average daily excretion of 20–30 g in a healthy individual (Guyton & Hall, 2016).

Excessive accumulation of urea in the blood, often due to renal failure or metabolic disorders, leads to a pathological condition called uremia, characterized by nitrogen toxicity that can affect various organs (Murray *et al.*, 2018). Blood urea nitrogen measurement is thus an

***Corresponding Author:** Al Cherif Hamid Mahamat

important clinical parameter for assessing renal function and protein metabolism (Kraut & Madias, 2017).

In our study, measuring urea levels allows us to monitor the impact of different diets on the renal function of young rats. Significant variations may reflect either an alteration in glomerular filtration or a modification of protein metabolism induced by the quality or toxicity of the ingested meal (Atchade *et al.*, 2019).

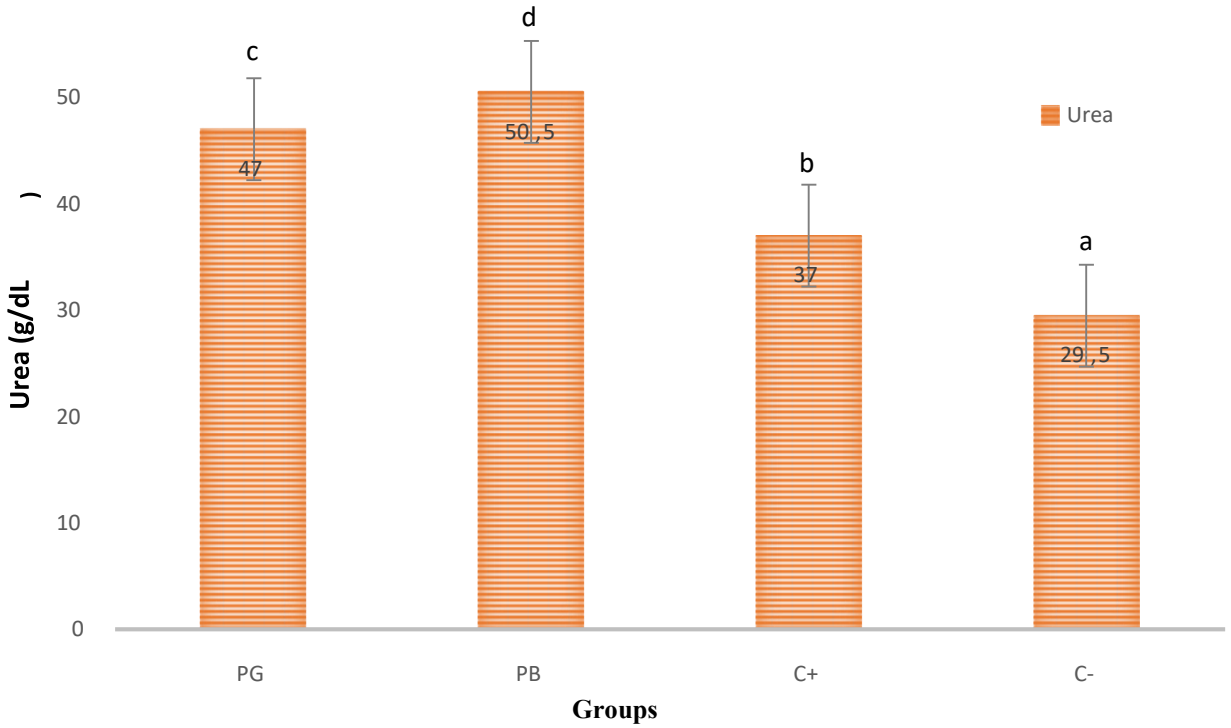

Figure 5: Influence of different diets on serum urea concentration

Serum urea concentrations measured in animals fed the different diets were as follows: 50.5 ± 2.12 mg/ dL for the boiled meal (PM) group, 47.0 ± 65.65 mg/ dL for the roasted meal (RM) group, 30.0 ± 0.0 mg/ dL for the casein-fed group (C+) and 29.5 ± 3.53 mg/ dL for the negative control group.

A significant increase in blood urea was observed in rats fed treated meal, particularly in the PB group, whose values significantly exceeded those of the casein group. This significant increase in blood urea suggests probable renal dysfunction, induced by the toxicity of boiled meal. This phenomenon could partly explain the mortality observed in the group receiving raw meal (PC).

Similar results were reported by Mugendi *et al.* (2010), who observed increased urea levels in chickens fed unpretreated *Mucuna seed meal, highlighting the negative impact of* antinutrients and toxins on kidney function.

c) Transaminases (ALT and ASAT)

Transaminases, also known as aminotransferases (ALAT and ASAT), are enzymes involved in the transfer of an amine group (-NH_2) from an amino acid to a ketone function (CO) of a ketonic acid. They play a key role in amino acid metabolism. Transaminases are the most frequently used markers for detecting liver damage. In the event of liver cell damage, these enzymes are released into the bloodstream, leading to an increase in their serum concentration (Steven *et al.*, 1994).

Increased serum ALAT and ASAT levels are therefore considered a sensitive indicator of liver damage, whether of toxic, infectious or nutritional origin (Giannini *et al.*, 2005). In this study, monitoring these enzymes allows us to assess the impact of different diets based on *Pachira oilcakes. insignis* on the liver integrity of animals. An abnormal elevation could reflect the presence of toxic compounds or antinutrients in the cakes, which could induce hepatic stress or cellular damage (Ozer *et al.*, 2008).

***Corresponding Author:** Al Cherif Hamid Mahamat

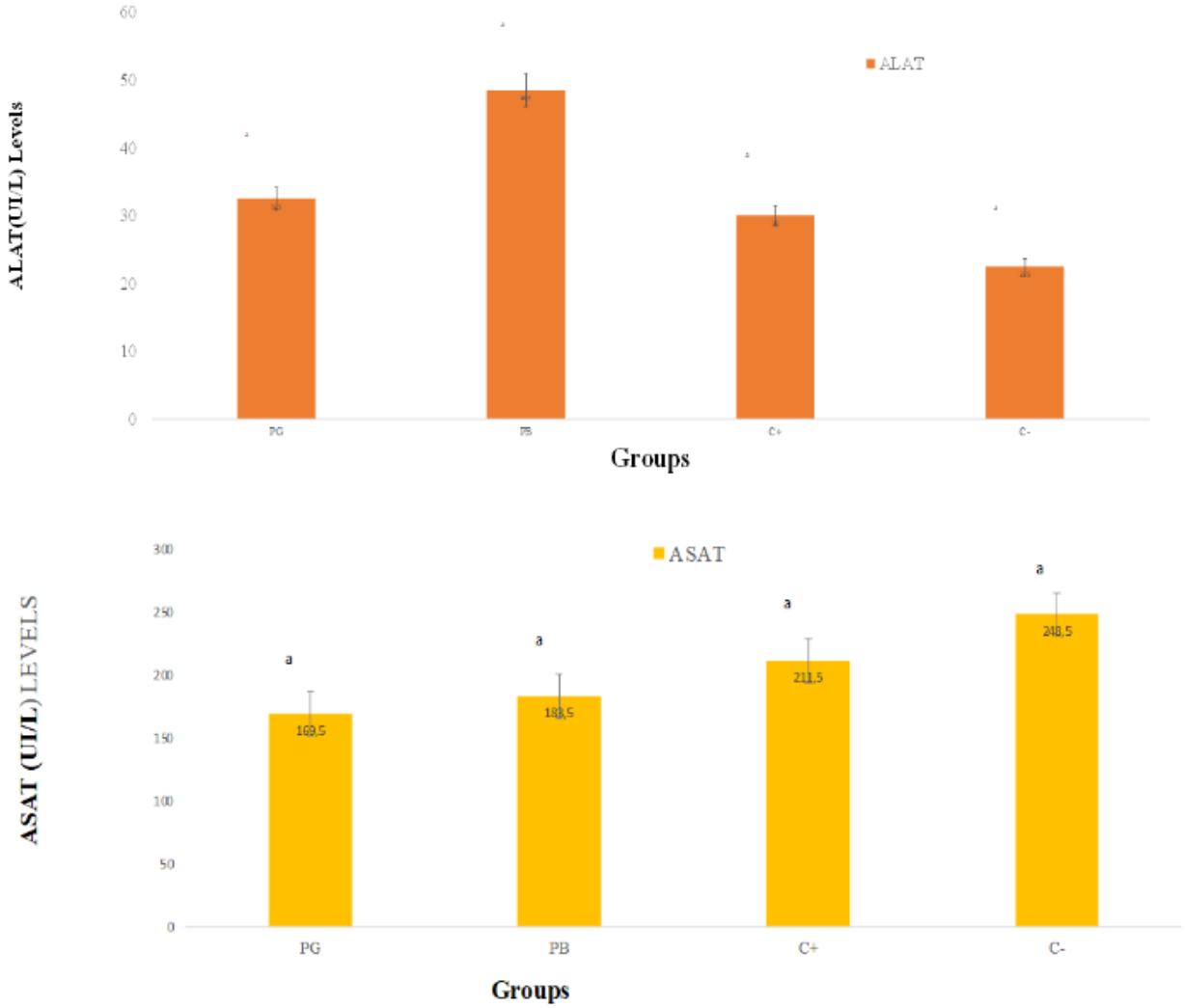

Figure 6: Influence of different diets on serum transaminases

Figure 13 shows the ASAT levels measured in the blood of the experimental groups fed with *Pachira cakes grilled (PG) and boiled (PB) insignis* , respectively 169.5 IU/L and 183.5 IU/L, compared to the casein-fed control group (C+) with a rate of 211.5 IU/L. Regarding ALAT, values of 32.5 IU/L for the PG group and 48.5 IU/L for the PB group were recorded, compared to 30 IU/L in the reference group.

Statistically, these differences are not significant (P = 0.05). However, the relative elevation of transaminases, although moderate, suggests potential liver dysfunction related to the consumption of *Pachira oilcakes. insignis*. This increase could reflect a slight damage or hepatic stress induced by certain compounds present in the cakes, particularly in boiled cake.

Body organ indices

Table 3 shows the weights of the different organs of the rats measured at the end of the experiment.

Table 3: Biometric indices of organs

	Heart	Liver	Kidneys	Testicles
TPG	$^{0.05}$ ab	4.68±0.32 ^{b}	0.45±0.09 ^{b}	0.41±0.19 ^{a}
TPB	0.45±0.03 ^{ab}	4.76±0.39 ^{c}	0.53±0.02 ^{c}	0.35±0.10 ^{a}
TPC	0	0	0	0
C+	0.39±0.02 ^{a}	3.55±0.35 ^{a}	0.39±0.02 ^{a}	0.56±0.43 ^{a}
C-	0.55±0.14 ^{b}	5.75±0.67 ^{d}	0.58±0.07 ^{d}	0.70±0.13 ^{a}

TPG = Pachira Crab grilled; TPB = Pachira crab boiled; TPC = Pachira Crab raw; C+= Positive control; C-= Negative control

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Changes in relative organ mass, or organ indices, are important indicators of toxicity. A significant increase in these indices after ingestion of a substance suggests potential toxicity (Raza *et al.*, 2002; Teo *et al.*, 2002).

According to the data in the table, organs such as the heart, liver, and kidneys of the casein-fed rats showed the lowest indices, with a cardiac index of 0.39 ± 0.02, for example, compared to the other groups. Analysis of variance revealed that there was no significant difference ($P < 0.05$) between the cardiac indices of the different groups tested, indicating that the diets did not affect cardiac function. Specifically, the groups receiving the roasted (TPG) and boiled (TPB) cakes showed similar cardiac indices, but higher than those of the negative control group (0.55 ± 0.14). This increase in the negative control group is probably related to the weight loss due to the total absence of protein in their diet.

Concerning the liver, a very highly significant difference ($P < 0.001$) is observed between the groups. The casein group has the lowest liver index (3.55 ± 0.35), while the TPG and TPB groups display higher indices, respectively 4.68 ± 0.32 and 4.76 ± 0.39. This increase reflects hepatic hypertrophy, a sign of poisoning of this vital organ linked to the consumption of *Pachira* cakes. *insignia*.

For the kidneys, a highly significant difference ($P < 0.01$) was also noted. The negative control group showed marked renal hypertrophy (0.58 ± 0.07) compared to the casein group (0.39 ± 0.02). The TPG and TPB groups also showed renal hypertrophy, suggesting renal toxicity induced by these diets. Finally, testicular indices were similar in all groups ($P < 0.05$), indicating that this organ appears to be spared from any form of diet-related toxicity.

Pachira insignis cakes, although potential protein sources, can induce toxic effects at the hepatic and renal levels, requiring particular vigilance regarding their dietary use.

CONCLUSION

This study on the nutritional properties of *oilcakes and proteins Pachira insignis* has allowed to better characterize certain physicochemical aspects of this cake, recognized as an interesting source of proteins and carbohydrates. The processing methods applied, in particular roasting and boiling in water, have made it possible to reduce the content of antinutrients present in the cake. However, despite these treatments, *Pachira insignis* cakes exhibit clear toxicity, characterized by kidney and liver hypertrophy, as well as increased liver enzymes (AST, ALT) and blood creatinine. This toxicity also led to animal mortality under certain experimental conditions. In view of these results, it would be wise to identify and develop more effective treatment processes to minimize antinutrients and toxic compounds, to conduct a thorough toxicity study including detailed biochemical and histological analyses of target organs, to perform histological sections of the organs to better understand the mechanisms of intoxication, as well as to extend the experiments to female rats to evaluate possible sex-related differences. These perspectives will help improve the safety and nutritional value of *cakes Pachira insignis*, with a view to their potential use in animal or human food.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this article.

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