Comparative analysis of two production methods of *Daddawa* base on traditional softening agents (*Kanwa* and *dalang*) *Comparative softening process of Parkia biglobosa seeds.*

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Abstract: *Daddawa* is a traditional condiment obtained by cooking and fermentation of African locust bean seeds (*Parkia biglobosa* Benth). The cooking process is very long (12-48 h), very difficult and time consuming. Putting in place a rapid and standard method of cooking by study the softening parameters (amount of *alkali*, introduction time of *alkali* in cooking liquid) at 96°C would perform his production. In the aim to evaluate the specific softening time, the studies of some softening parameters, the fermentation time and the physicochemical characterization of daddada produced by using *dalang* and *kanwa* as softening agent were realized. From these studies it was noted that *dalang* at the level of 0.75% (W/W) and *kanwa* at the level of 1% (W/W), introduced after 60 min of cooking facilitates the softening and permits to reduce the cooking time. The maximum of dehulling (100%) was obtained after 3 h. In this case, standardization process of softening is possible by using 48 h of soaking; 0.75% of *dalang* or 1% of kanwa; 60 min introduction time of *alkali*. These specifics parameters have contributed to reduce the specific softening time at 3 h. After three days of fermentation, the results in proteins and carbohydrates digestibility and mineral elements were good when *dalang* is used as softening agent. When *kanwa* is used proteins, lipid, carbohydrates and energy are important than in *dalang* solution. In general there is no significant difference in the softening methods when *dalang* or *kanwa* is used. But the physicochemical parameters are significantly difference ($p \leq 5\%$)

Keyswords: Kanwa, dalang; dehulling, Parkia biglobosa seeds, softening parameters, specific softening time.

Abbreviation: **PDS**, percentage of dehulled seeds; **SST**, specific softening time; **DAK:** *Daddawa* produced from seeds cooked in *kanwa* solution; **DAW:** *Daddawa* produced from seeds cooked in pure distilled water*;* **DAD:** *Daddawa* produced from seeds cooked in *dalang* solution; **CC:** cooked cotyledons; **DN:** *Daddawa* bought in Ngaoundere market

Introduction

Daddawa is a food condiment produced by alkaline fermentation of African locust bean seeds (*Parkia biglobosa* Benth). This condiment is popular and consumed by several ethnic groups (*dii; foulbé; haussa; laka; mafa; mboum; mundang; ngambaye*) in the Northern part of Cameroon.[1][2] It constitutes an important source of proteins (34-54%) and lipids (19-21%)[2][3] necessary to improve African diets (flavouring soups and stews), which are nutritionally poor and calorifically unbalanced. Many difficulties are encountered during the transformation of locust bean seeds into *daddawa*.[4] The most difficult step is the cooking and dehulling of locust bean seeds using the traditional method. This method happens to be laborious and time consuming due to the hard seed coats, which must be dehulled after a long cooking time (24-72 hours).[2][5] At maturity, the coat is dry thereby rendering the access to the cotyledon difficult, leading to long cooking time (12-48h). Dried bean seeds are cooked in an alkaline solution in order to soften the seed cell walls and the starchy granules within them. Cooking the seeds to the point at which the coat splits depends on its composition and that of the cooking medium.[6] It is therefore important to characterise the cooking conditions of the seeds. To overcome the cooking and dehulling difficulties for processing and development of improved methods using suitable techniques, the cooking conditions of the seeds need to be mastered.

In the other side, *daddawa* quality is determined by its nutritive value. Which is impacted by the softening process.Women who produce *daddawa* are using different techniques such as alkaline cooking agents (*kanwa*, *dalang* etc…). In north Cameroon *dalang* and *kanwa* are always used as softening agent. When regarding seeds like bean, *kanwa* is always used as softening agent. In contrally *dalang* is used to soften meat and some vegetables. But when come to soften *P. biglobosa* seed, which is used in the production of *daddawa*, women prefere to use wood ash which is the fundemental element used in *dalang* production. They seldom use *kanwa*; when they do so, these softening agents are usually poorly used due to ignorance.[1] It is therefore important to look at how a softening agent could influence the process of softening and dehulling of *P. biglobosa* seeds.

Putting in place a standardised softening process for African locust bean seeds during the production of *daddawa* thus becomes crucial. For this, it is indispensable to study the softening parameters which would facilitate softening and reduce cooking time. This will be done using *kanwa and dalang*. This study was carried out with the aim of identifying suitable processing methods of cooking, which could rapidly, effectively and efficiently reduce cooking time and enhance the nutritional value of the product. The Specific Softening Time will be determined hence. To evalute the nutritional quality, fermentation and physicochemical characterisation of the *daddawa* condiments produced from seeds cooked in *kanwa and dalang* solution and those cooked in distilled water will be comparatively assessed. This physicochemical characterisation of the *daddawa* condiments produced will enable one to know the chemical, nutritional properties of both *daddawa* condiments. It will consist more precisely in verifying whether the optimum cooking and fermentation conditions put in place permit to obtain acceptable physicochemical and nutritional properties. Indeed, many parameters (moisture content, cooking time, ambient temperature and air hygrometry) influence fermentation conditions and the obtainability of the flavour, odour and texture sought.[2] According to Shao[7]; Doumta and Tchiégang[1][2] poor fermentation produces a product which is unfit for consumption.

The general objective of this study is to ameliorate the traditional production process of *daddawa* by putting in place a standardised production process base on softening agent.

I. Material and methods

I.1. Seed sample

Seeds of *Parkia biglobosa*, were bought at the "Grand marché" of Garoua. The seeds selected for experiment were undamaged seeds, free from any foreign material. The locust bean seed used was physically characterised as: moisture content (7.33% mc), mass (0.35 to 0.55g), length (1.18 to 1.50 cm), width (0.86 to 1.10 cm) and thickness (0.42 to 0.50 cm). The primary investigation showed that this sample had lower amounts of seed coat (less than 30%) and higher percentage of dehulled seeds (up to 70%) when cooked in distilled water for 10 hours.[1][2] As such, different parameters were monitored and suitable analyses aimed at attaining our specific objectives were conducted. Prior to these investigations, the seeds were sorted, cleaned and appropriate masses weighed. *Kanwa* and *dalang* purchased at the "Bantaï Market" in Ngaoundere (Adamaoua Cameroon) were used as softening agents.

I.2. Assessment of softening parameters of *Parkia biglobosa* seeds

Damaged seeds and foreign matter were sorted out manually. Their presence will influence adversely the results hence, wrong interpretations. Suitable seeds were washed with tap water and rinsed with distilled water so as to remove any foreign matter (dust) stuck to the seed coat.

I.2.1. Analysis of *softening agents*

Cooking time varies with the composition of cooking liquid.[6] The alkaline substance used were *kanwa* and *dalang*. they are commonly used as cooking aid, to facilitate the cooking process in the Northern part of Cameroon and serve the purpose of enhancing the flavour and tenderising; hence reducing the cooking time of locust bean seeds. They are mainly used as a softening agent in cooking dry seeds. *Kanwa* and *dalang* salts are used in the form of ash. This ash was obtained after incineration at 550°C for 3 hours in an incinerator of trade mark PROLABO. The proximate mineral composition of ash was determined.

I.2.2. Soaking of seeds

In the traditional preparation of *daddawa*, this step is usually neglected**.** Doumta and Tchiégang[1] studied the effect of soaking on African locust bean seeds prior to cooking and found that soaking reduces the cooking time of locust bean seeds (by 20%) during *daddawa* condiment production. They came to the conclusion that the optimum soaking time is two days. It is in this lamplight that the seeds were soaked in distilled water for two days. It is worth noting that the water was replaced after every 24 hours to avoid fermentation. The seeds were soaked at room temperature (25±2°C).

I.2.3. Influence of the amount of alkali on percentage of dehulled seeds

This study was carried out to determine the optimum amount of alkali which will best reduce the softening time of locust bean seeds during the cooking process, thereby reducing the cooking time.

500g of seeds each were removed from a whole sample then divided into five portions of 100 g each, corresponding to 0; 0.5; 0.75; 1; 1.5 % w/w (mass of alkali/100 g of seeds) respectively. The samples were soaked for 48 hours and cooked with distilled water in 5 different pots accordingly for 2 hours. Since the task here consisted in determining the influence of the amount of alkali in reducing cooking time in the process of *daddawa* production, the alkali was introduced after an hour of cooking.

NB: The cooking process starts effectively when the water starts boiling. It is at this instant that the timer is launched.

The percentage of dehulled seeds (PDS) was determined after 2 hours of cooking. At the end of this investigation, the amount of alkali which produced the best PDS was adopted as optimum amount of alkali for further investigation.

I.2.4. Influence of the introduction time of alkali on percentage of dehulled seeds

This study was carried out to determine the cooking time at which it was suitable to introduce the alkaline substance in the cooking medium.

500 g of soaked seeds were removed from a whole sample and divided into five portions of 100 g each, labelled 0; 30; 60; 90; 120 minutes, respectively corresponding to the time of introduction of the alkaline substance in the cooking medium when cooking starts. The introduction time was respected for each sample. The amount of alkali to be introduced is the optimum obtained from the previous investigation. The rate of dehulled seeds was expressed as a percentage after 2 hours 30 minutes of cooking. This time was adopted to appreciate the PDS. The introduction time at which the PDS is highest was adopted for use in the next investigation. This is the optimum introduction time.

I.2.5. Determination of the Specific Softening Time

The study of physicochemical properties of softening is aimed at determining the specific softening time (SST). The SST corresponds to the time at which the PDS is maximum. This is achieved at optimum softening parameters. This study lies on the assessment of the PDS when optimum softening parameters are implemented in the cooking process.

Of course, in order to demonstrate the relative efficiency and effectiveness of our alkaline cooking process compared to the traditional process, three cooking medias were used: distilled water, alkaline solution of kanwa and dalang.

This investigation was carried out by implementing the optimum softening parameters studied above.

1800 g of seeds (divided into 18 portions of 100 g each, 6 of which were processed in distilled water 6 and the other 12 in alkalines solutions (6 in *kanwa* and other in *dalang*) were soaked for two days in distilled water (obtained from previous studies of Doumta and Tchiégang[8] on soaking time) and cooked in distilled water and in an alkaline solution of *kanwa* in the proportion 2/5 (mass of seed/Volume of cooking liquid) in aluminium pots. When the cooking liquid reduced, boiled water was added to avoid decrease in temperature. **T**% w/w of *kanwa or dalang*-mass of alkali/100 g of seeds- (amount of alkali to be introduced in cooking medium obtained from the previous study of the influence of the amount of alkali on softening of *P. biglobosa* seeds) was added **X** minutes after the water in the aluminium pot had boiled (time obtained from the previous study of the influence of the introduction time of the alkali on softening).

The samples were cooked for 1; 2; 3; 4; 5 and 6 hours according to the corresponding time. After cooking a sample of 100 grains was randomly taken from the pots and the PDS was determined. After cooking and cooling, excess cooking liquid was drained out while the seeds were dehulled manually. Removal of the seed coat was achieved by rubbing the seed coat between the palms of the hand. The operation was repeated until seed coats were totally removed. The cooking time for which the PDS was maximum was noted as the SST. This investigation permits to appreciate the softening process which is to be standardised.

Specific Softening Time (SST) is defined as the time at which PDS is maximum and observe no variations. SST is drawn from the kinetics of PDS with respect to particular pertinent and precise softening parameters which influence softening time.

Percentage of dehulled seed (PDS) : It helps determine the point at which the softening of the seeds is maximum.

To determine the PDS, 100 cooked seeds were randomly selected following the method described by Dutta *et al*.[9] For each experiment, the ratio between the number of cooked and dehulled seeds over 100 cooked seeds randomly selected was determined. The established relationship is as follows:

$$\boldsymbol{PDS} = (\boldsymbol{cooked\ and\ dehulled\ seeds}/\boldsymbol{cooked\ seeds\ randomly\ selected}) \times \mathbf{100}$$

The result is an average of 3 trials.

I.2.6. Pre-treatment of cotyledons before fermentation

This consisted in further cooking the cotyledons in distilled water at boiling point for an additional 1 hour before fermentation of these cotyledons. The cotyledons were cooled using cold water and rinsed. The cotyledons were then fermented before the *in vitro* digestibility tests of proteins and carbohydrates were carried out to determine the best time for complete fermentation with quality nutritional properties. Physicochemical characterisation of the fermented products -*daddawa*- then proceeded.

I.3. Assessment of biochemical and physicochemical parameters of the *daddawa* condiments produced

I.3.1. Fermentation

The cotyledons were placed in filter papers in iron vessels and spontaneously fermented for 4 days in a fermentor (Fisher Bioblock Scientific) at 25°C to maintain conducive conditions for fermentation. NaCl crystals were spread on the cotyledons to minimise fungal attack. Samples were taken using a clean spatula from the "zeroth" to the fourth day of fermentation to assess changes in biochemical parameters of proteins and carbohydrates so as to determine the best fermentation time for good *daddawa.*

Moist samples of *daddawa* were dried in an oven for 48 hours at 40°C before grinding using a mortar and pestle. Flours obtained were used for the biochemical analyses.

I.3.2. Assessment of biochemical parameters

I3.2.1. *In vitro* digestibility of proteins

1g of ground *daddawa* was mixed with 35 mL of pepsin (1.5 g/L) prepared in phosphate buffer (0.1M) at pH 2. After incubation at 37°C for 2 hours in a water bath, the residue was washed with 10 mL of phosphate buffer (pH 7) and centrifuged again at 4000 rev/min for 15 min. The residue was mixed with 35 mL of papain (1.5 g/L) prepared in phosphate buffer (0.1M) at pH 8 and incubated at 37°C for 1 hour. At the end of incubation the mixture was centrifuged at 4000 rev/min for 15 minutes. The sample was then treated with trichloroacetic acid (10 ml) to remove the rest of free protein. The whole preparation was centrifuged at 4000 rev/min for 15 minutes. The protease activity was stopped by incubating the preparation in a water bath at 100°C for 10 min. Nitrogen in the supernatant was determined by the method of Lowry *et al.*[10]

I.3.2.2. *In vitro* digestibility of carbohydrates

Ground *daddawa* (1g) was mixed with 35 ml of pancreatic amylase (1.5 g/L) followed by an incubation in a water bath at 37°C for 2 hours. At the end of incubation, amylase activity was stopped by incubating the preparation in a water bath at 100°C for 10 minutes. The mixture was centrifuged at 4000 rev/min for 15 minutes and the residue washed with phosphate buffer (10 mL) (pH 7) and centrifuged again at 4000 rev/min for 15 minutes at room temperature. The supernatant is recovered and the free sugars titrated according to the Fischer & Stein[11] method.

I.3.3. Determination of the amount of some principal nutritional compounds

The determination of the amount of some principal nutrients is an important step in the characterisation of products produced in the laboratory. This brings a plus value in ameliorating the production process.[2] The analyses were done on *daddawa* produced from seeds cooked in an alkaline solution of *kanwa, dalang* and those cooked in pure distilled water.

The determination of dry matter, Total ash are quantified by the method described in AOAC.[12] The lipids are extracted using the Soxhlet method according to the Russian method described by Bourely.[13] Total sugars were titrated according to the method described by Fischer and Stein.[11] Total nitrogen is determined after mineralisation of the samples according to the method of Kjeldahl[14], and titration according to the colorimetric technique of Devani *et al.*[15] The energy value (kcal/100g) was estimated by multiplying the percentage of crude protein, crude lipid as well as carbohydrate by 4, 9 and 4 respectively as conversion factors.[16] This ash solution is used to test for the presence of minerals by Atomic Absorption Spectrometry. The following metals were tested for; K, Na, Ca, Fe, Zn.

I.4. Statistical analysis

Analyses were carried out in triplicate. A completely randomized design was used to analyse percentage of dehullind data. The mean values for all parameters were examined for significance by analysis of variance and when significant differences ($P \leq 0.05$)

was observed. Mean separation was accomplished by Duncan's multiple range test using STATISTICA software.[17] Graphic representations were made using the Sigma Plot graphics package.[18]

II. Results and discussion

II.1. Proximate composition of *kanwa* and *dalang*

The proximate composition of *kanwa* presented in **Figure 1** showed the distribution of mineral contents.

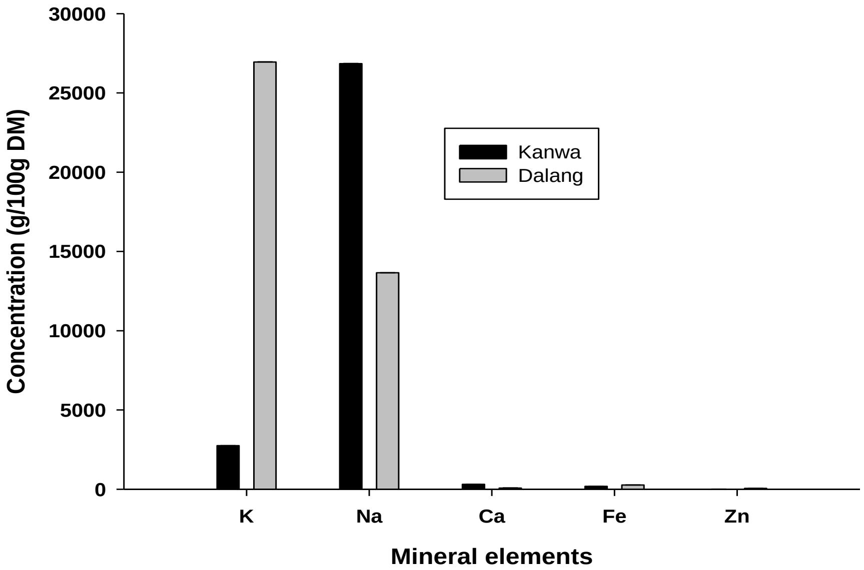

Figure 1: Mineral contents of *dalang and kanwa*.

From these results, it is observed that sodium (26.85 ± 1.36 g/100g) is the most present element follow by potassium (2.75 ± 0.98 g/100g) in *kanwa*. Whereas calcium (311.14 ± 1 mg/100g), iron (181.01 ± 0.80 mg/100g), zinc (0.53 ± 0.06 mg/100g) follow the order. The mineral composition of *kanwa* assessed seem to be in contradiction to the *dalang* composition. For *dalang*, essential elements are in order potassium (26.95± 2.41 g/100g); sodium (13.65 ± 0.98 g/100g); iron (264.01 ± 0.80 mg/100g), calcium (76.14 ± 1 mg/100g), zinc (58.33 ± 0.06 mg/100g). The *dalang* composition is similar to the composition found by Doumta and Tchiégang.[1][2] The composition of these salts could help in reducing the softening time by enhancing the pH of cooking solution. Previous studies have shown that cooking time varies with water composition as reflected by its source, and deionised water tends to result in faster cooking time than either tap water or borehole water which is usually rich in both divalent and monovalent cations.[19] According to Kristin[6] and Kimball[20] softening can be sped by making the water more alkaline. Traditionally in Northern part of Cameroon, *kanwa* and *dalang* are used to soften the seeds and they are added to the cooking liquid after a long period of cooking. Hence the speed of the softening depends on the period of adjunction and the quantity of softening agent.

II. 2. Amelioration of softening methods

Amelioration is focus on four parameters: amount of alkali; introduction time of alkali in cooking milieu; specific softening time and biochemical analysis.

II.2.1. Influence of the amount of *kanwa* and *dalang* on the softening

The PDS is plotted against percentage of alkali introduced. **Figure 2** presents the influence of the amount of *kanwa* and *dalang* on the percentage of dehulled seeds.

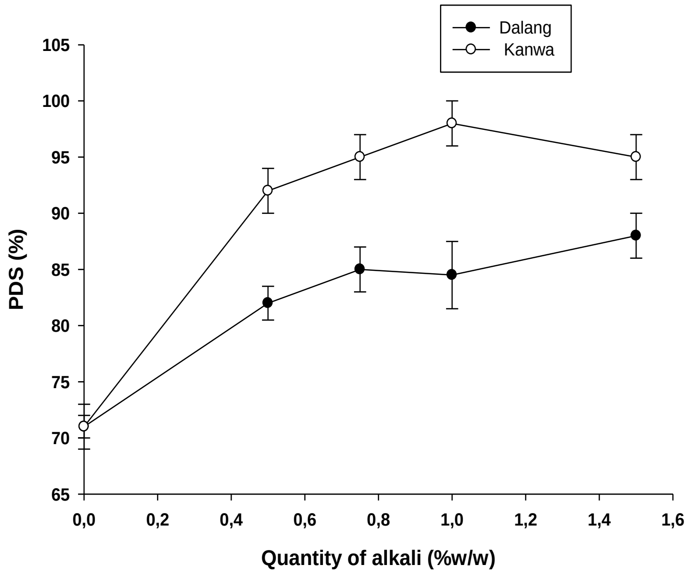

Figure 2 : Effect of the amount of *kanwa* and *dalang* on the percentage of dehulled seeds.

Cooking with alkaline salt significantly altered the seed coats and increased the PDS. Means of dehulled seeds at 0; 0.5; 0.75; 1.0; 1.5% of *dalang* and *kanwa* varied between 71.5±2 to 88.0±2% and 71.5±2 to 98.0±3% respectively. The PDS for the seeds cooked with 0% *alkali* is 71.5±2%. This gradually increases as the % *alkali* increases up to the peak, 88.0±1.41%, at 0.75% *dalang while* 98.0±3%, *at* 1% *kanwa*. The peak is the point at which the PDS is maximum. The effect of *kanwa* and *dalang* on cooking was not significantly different ($p < 0.05$) with respect to the amount of alkali used according to statistical analysis. Therefore, 1% *kanwa* and 0.75% *dalang* are optimum for maximum yield of dehulled seeds after cooking for 2 h 30min. This pourcentage is closed to value (0.75%) obtained with *dalang* in the same condition.[2][8]

The PDS increases with the amount of *alkali* introduced up to the optimum, due to the combine effect of pH and temperature of the seed coat. The presence of the alkali contributes in increasing the ionic strength of the cooking medium and under the effect of pH and temperature, the proteins liberated by breakage of hydrogen and peptides bonds will form protein-protein; protein-water or protein-ion complexes which result in the denaturation of the seed coat.[8] Consequently, the cooking solution will be able to diffuse through/into the seed coat and soften the seed which reduce cooking time and ease dehulling. It would have thus, been expected that the optimum amount of *alkali* to be 1.5% but a drop in PDS is noticed at this concentration. This could be accounted for by the fact that above the optimum % *alkali*, the complexes formed are such that they instead block the pores of the seed coat inhibiting diffusion of the cooking solution into the seed coat hence, softening time is increased and the PDS is decreased. In other words the “hard to cook” phenomenon is responsible for this by the formation of inhibiting complexes of ions present in the solution with the proteins of the seed coat.

All in all, the action of *alkali* provokes the formation of holes in the seed coat and facilitated the transfer of water from the cooking solution to the cotyledon. This transfer would induce the swelling of seed and the breakage of the inter and intra cellular chains.[21]

Base on results obtained with different quantities of alkali used, the statistical analyses reveled that there is significant difference different ($p < 0.05$) between softening in *dalang* and *kanwa*.

II.2.2. Effect of the introduction time of *alkali* in the cooking liquid

Knowing which amount of *alkali* to introduce is important as well as knowing the time at which it should be introduced in order to have maximum PDS. The introduction time of the optimum *alkali* (1% *kanwa* and 0.75% *dalang)*, for which PDS are maximum after cooking for 2h30 min, were taken as the optimum time at which the optimum quantities of *alkali* to introduce in cooking liquid. **Figure 3** shows the influence of the introduction time of the alkaline salt in the cooking liquid on softening of the seeds.

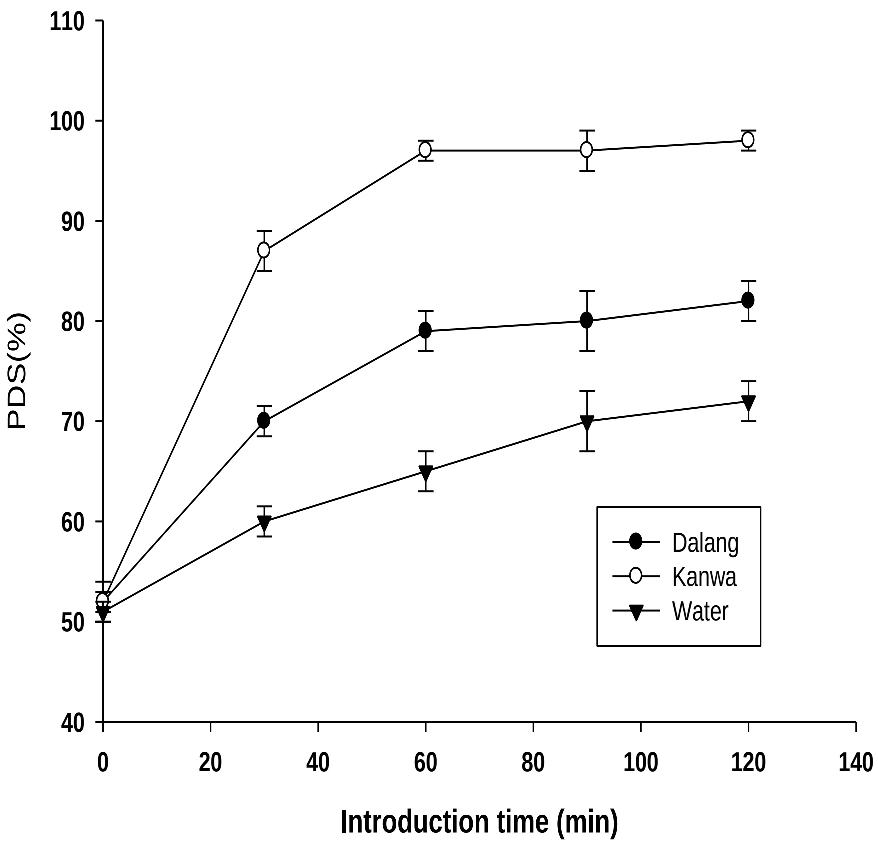

Figure 3: Influence of the introduction time of *dalang* and kanwa in the cooking liquid.

The time at which the *alkali* is added, shows significant differences on the PDS. When it was added earlier (“at time zero”) the PDS were 51.5±7% (*kanwa /dalang*). This number reached to maximum of 97.25±2.22 and 97.25±2.75 respectively at 30 and 120 min in *kanwa*, while in *dalang*, the maximum was 85% obtained at 90 and 120 min. Base on these obervations it seems that *kanwa* improve the softening process very father than *dalang*. Statistical analyses show that the sofetning methods (water, dalang and kanwa) are different ($P \leq 0.05$) base the PDS. The low value observed when, the softening agent was added earlier, could be due to the formation of complex structures. Divalent cations such as calcium are bound by pectic substances within the middle lamellae of the bean cotyledon, forming calcium pectates.[22] Calcium pectates are insoluble and resist cell separation during cooking. Therefore, beans that are cooked in water that contains high divalent cations have a longer cooking time when cations are in the cooking liquid at the beginning of the cooking.[2][23] This could explain why the percentages of dehulled seeds were lower at zero and 30 min of introduction time. In this study, distilled water used at the beginning of the process, contributes to move out the pectic substance content in seed coat and modified the cell wall structure. When the softening agent was added at this moment, divalent cations such as calcium are bound to the free bonds left by the removal of pectic substances and break the inter and intra chain bonds. This contributes in lowering the cooking time. For these two alkalis *kanwa* presents the higher quantity of Calcium (311,14 mg /100g) than *dalang* (76 mg/100g). This can explain the fact that PDS is faster with *kanwa* than *dalang*.

According to Kristin,[6] these actions would work to slow the softening of beans in four different ways by stabilizing hemicellulose, strengthening cell wall, slowing the swelling of starch granules and cross-linking pectins. Initially, when placed in distilled water, the dried bean seeds can only absorb water through their pores. At the right time of introduction of alkali (30 or 60 minutes of cooking), the seed coats expand and become hydrated. At that point, water cations can move into the bean through the hilum and the entire seed coat surface. Then the seed becomes soft and dehulls easily. Between adding *alkali* at 60, 90 or 120 minutes they was no significant differences ($P \leq 0.05$) when the percentage of dehulled seed is concerned. This means that, introduction time of *alkali* in cooking liquid should be up to 60 min.

II.2.3. Identification of the Specific Softening Time

Figure 4 indicates the influence of optimum alkaline cooking on softening compared to cooking in distilled water without *alkali*.

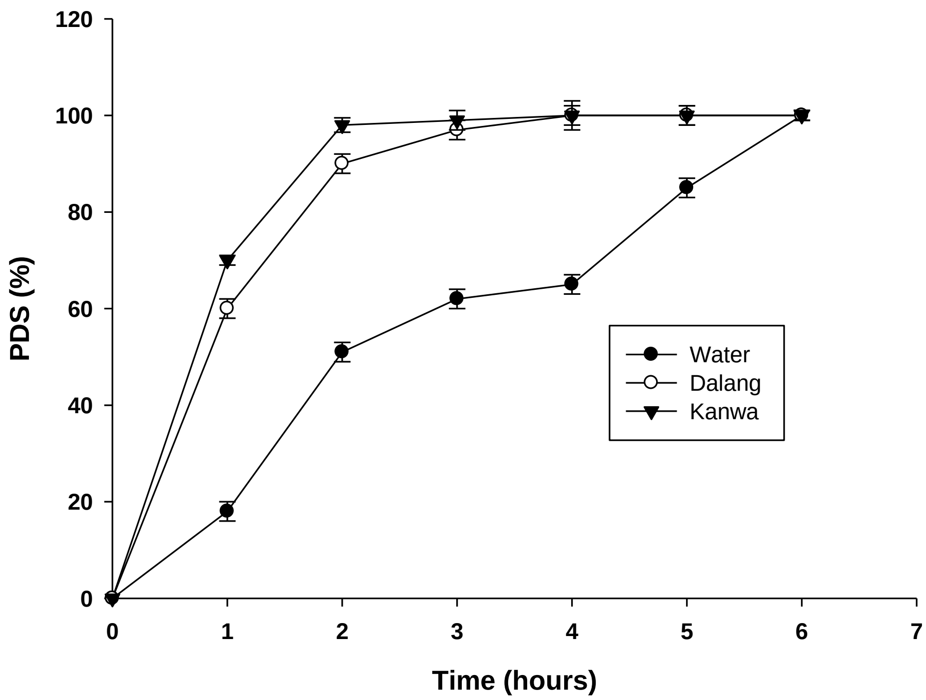

Figure 4: Effect of combined parameters identified on softening with the comparison to cooking in distilled water.

For both treatments (alkaline solution and distilled water) the results showed an increase in the PDS. Softening significantly increased ($p<0.05$) during the three first hours when *kanwa* and *dalang* were used. In fact, the PDS at the first hour of cooking was 54%. After 3 hours, 100% of seeds were dehulled when *kanwa* and dalang were used. This value was almost obtained (100%) after six hours of cooking with distilled water. The PDS after four, five and six hours of cooking using alkaline treatment showed no differences ($p<0.05$) between the PDS.

Base on these results the softening time reduced from 12 hours in traditional methods to 3 hours which corresponds to 75%. This result agrees with Kristin[6]who said, cooking with alkaline salt can reduce the cooking time by nearly 75%.

On the other hand, the PDS when cooking with distilled water shows differences with cooking time. These results are similar to Doumta and Tchiégang[2][8] who found the same results by using *dalang* as softening agent. According Kristin;[6] Doumta and Tchiégang,[2][8] cooking with alkaline salt can reduce the cooking time by nearly 75%. This is due to the presence of cations (Na+, K+) in the cooking liquid (*kanwa solution*) which work by helping the hemicellulose dissolve in cooking water and kick out the magnesium from the pectins in the cell walls and thereby making them more readily dissolvable.[21] The used of *kanwa* and *dalang* as base, shows the advantage of reacting faster; but it can also destroy partially the polysaccharides into simple sugars or uronic acid.[8]

According to Seymour & Gross[24] the higher diffusion of water in the alkaline cooked seeds contributes to weaken the seed coats with the loss of their firmness.[16] This loss of firmness leads to the loss of cohesion between different cells and provoke a disorganisation of the cell wall. This disorganisation of the cell wall plays the role of cohesive link and assures the vegetal integrity.[25] This observation could explain the higher PDS (100%) obtained with *alkali*. Hence, cooking time can be reduced, not by simply pre-soaking the beans for 48 h in water, but by the addition, at the precise time (60 min after boiling) and precise quantity of *alkali* (0.75-1%) to the cooking liquid. Between cooking with distilled water and alkaline cooking, the difference of the cooking time was about 3 hours. This corresponded to 50% of reduction of cooking time obtained with simply cooking and 75% of the traditional method.[2][8] Between *kanwa* and *dalang* there is not significant difference ($P \leq 0.05$) when regarding softening time. Therefore, the Specific Softening Time when *kanwa* or *dalang* is used at optimum softening parameters is 3 hours.

According to **Fig**. **4**, SST could be fixed at 3 h. At this time, 100% of dehulled seed was obtained using *kanwa and dalang*. But this time it is enough to produire a *daddawa* with good nutrives values?

II.3. Biochemical analyses

For all the following analyses, *daddawa* from seeds cooked in *alkali* solutions and *daddawa* from seeds cooked in distilled water were used so as to assess the effectiveness of *alkali* in ameliorating *daddawa* production. Digestibility tests of proteins and carbohydrates with respect to fermentation time will help in determining the best time for fermentation to be complete.

II.3.1. *In Vitro* Protein Digestibility

The digestion of proteins was done in presence of two proteases (papain and pepsin) which are protein hydrolases. These enzymes generally do not need coenzymes. They can be activated by cations. These endoproteases cleave peptides bonds found within the protein far from terminal C and N amino acids.[2]

Figure 5 shows the effect of alkaline cooking and that of cooking in distilled water on the *In Vitro* Protein Digestibility (IVPD) of the respective *daddawa* condiments produced with respect to fermentation time. This helps deduce the best fermentation time.

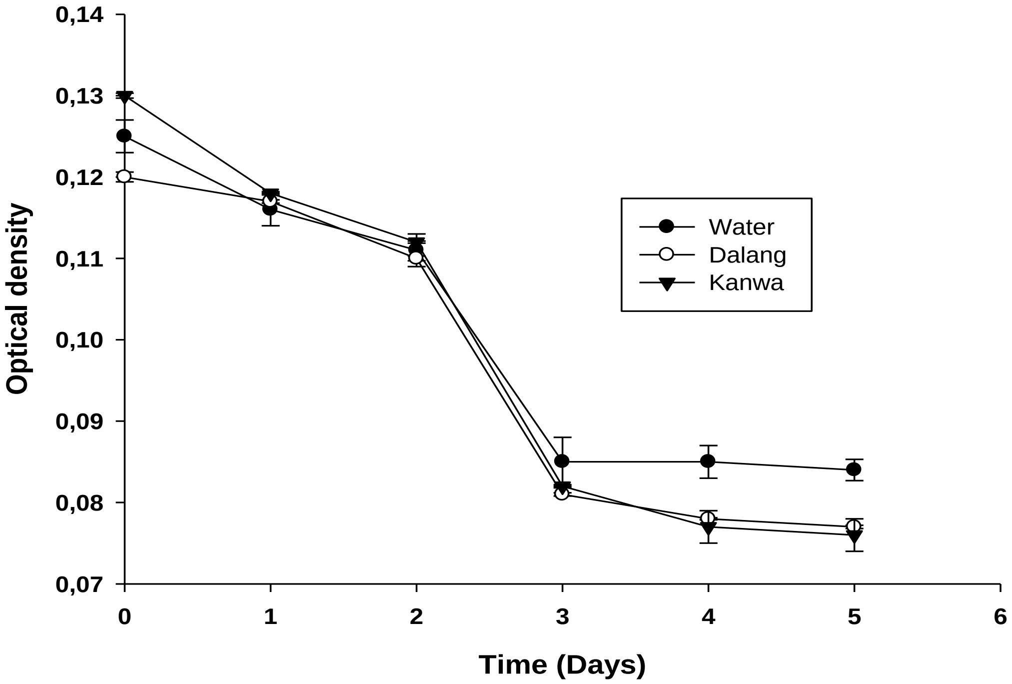

Figure 5: Influence of fermentation time on the IVPD of *daddawa* produced

The graph above show that the optical densities of *daddawa* condiments decrease with fermentation time. This could be explained by the fact that, as days go by, digestibility increases. It is also noticed that optical densities of *daddawa* from seeds cooked in *kanwa* solution are relatively smaller. The slope decreases because peptide bonds are broken hence a reduction in their numbers. This means that *kanwa* facilitates digestion of proteins more than *dalang*.

IVPD was significantly increased with fermentation time. According to statistical analysis (ANOVA), there is no significant difference ($p<0.05$) between the degree of digestibility of both *daddawa* fermented for 72 and 96 hours. It would be therefore be judicious to stop fermentation after three days since the aim is to produce *daddawa*. This fermentation time of 72 hours is in accordance with studies made by Shao.[7]

Protein digestibility of *daddawa* produced from seeds cooked in an alkaline solutions were higher than that of *daddawa* produced from seeds cooked in distilled water. The results reveal that alkaline cooking treatment using *kanwa* or *dalang* increase the digestibility of proteins.

II.3.2. *In Vitro* Carbohydrate Digestibility

Figure 6 shows the *in vitro* carbohydrate digestibility (IVCD) of *daddawa* produced from seeds cooked in an *kanwa* solution was higher than that of *daddawa* produced from seeds cooked in distilled water.

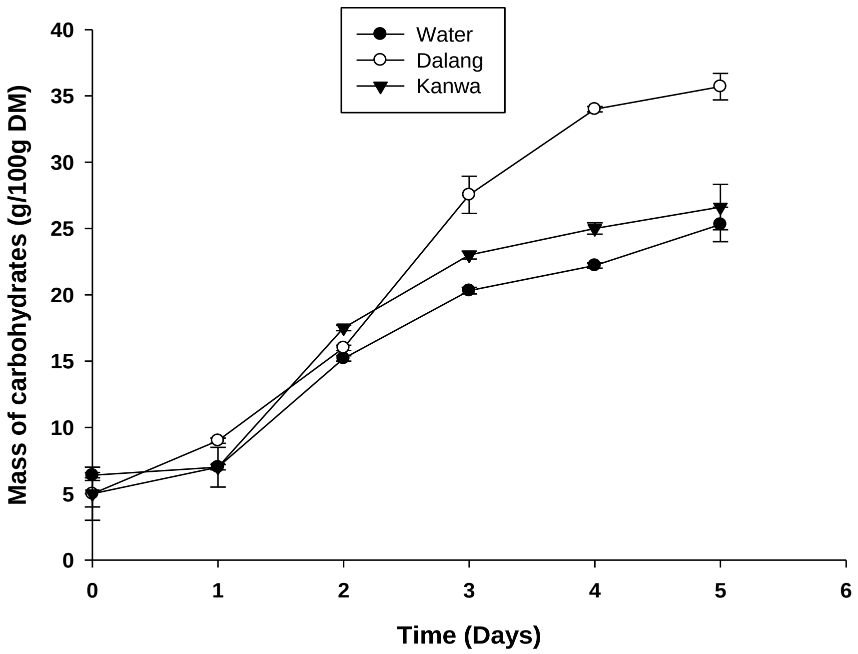

Figure 6: Influence of fermentation time on the IVCD of *daddawa* produced

The digestion of carbohydrates was done by α-amylase, a digestive enzyme classified under saccharidase which breaks down polysaccharides.[2] This enzyme is used for the catabolism of long chain carbohydrates such as starch, into smaller units.

Alpha amylase breaks α (1, 4) glycosidic linkages of amylase to give two molecules of maltose (disaccharide of α-glucose); It only attacks cooked starch.[2]

IVCD significantly ($p<0.05$) increases with the fermentation time. It has been suggested that difference in physico-chemical properties may not exist between starches cooked in distilled water and alkaline solution.[26] It appears that the softening agent improved starch digestion by increasing substrate accessibility to alpha amylase. This is more effective on *daddawa* produced with *dalang* than *daddawa* produces with *kanwa* after three days of fermentation. This is in agreement with the observations of Bernard *et al.*[27] and Ezeogu *et al*.[28] who estimated that softening agent ameliorate de digestibility of starch. According to statistical analysis, there is no significant ($p<0.05$) difference between the values of digested carbohydrates in *daddawa* obtained after 72 and 96 hours of fermentation for both treatments.

From the IVPD and IVCD, it is seen that after 72 hours fermentation is complete. The best fermentation time is therefore, 72 hours for good *daddawa*.

The desired state of fermentation of the condiments is indicated by the good quality of nutritive values which is characterized by overtones of ammonia produced as a result of the breakdown of amino acids during the fermentation.[29] The characteristic ammoniacal odour and flavour of condiments enhance the taste of traditional soups and sauces. This condiment serves as both flavour enhancer and nutritious non-meat protein in rural communities.

II.4. Physicochemical characterisation of cooked cotyledon and *daddawa*

After improving softening and fermentation parameters assessing physicochemical characterisation of cooked cotyledon and *daddawa* will help to appreciate the impact of amelioration process of the quality o*f daddawa*. **Table 1** shows the physicochemical characterisation of cooked cotyledon and *daddawa* used

Table 1: Physicochemical characterisation of cooked cotyledon and *daddawa* used (g/100g).

	CC	DAK	DAW	DAD	DN
Dry matter (g/100g)	97.38±0.42^{d}	95.13±0.12^{c}	95.3±0.22^{c}	89.16±1.90^{b}	84.73±0.85^{a}
Crude proteins (g/100g)	47.01±0.61^{d}	38.61±0.75^{b}	34.84±0.67^{a}	35.56±0.47^{a}	43.26±0.97^{c}
Total lipids (g/100g)	17.35±0.85^{b}	21.41±0.59^{c}	20.60±1.19^{c}	21.48±0.18^{c}	13.87±0.58^{a}
Total sugars (g/100g)	10.67±0.48^{a}	35.42±0.92^{d}	43.39±0.77^{e}	20.04±0.45^{c}	12.00±0.36^{b}
Energy value (kcal/100g)	386.78±7.40^{b}	489.57±1.62^{d}	498.32±11.1^{d}	415.72±5.3^{c}	345.87±0.1^{a}
Ash (g/100g)	10.50±0.31^{c}	2.07±0.14^{a}	1.07±0.21^{a}	5.76±0.25^{b}	6.01±0.98^{b}

DAK: *Daddawa* produced from seeds cooked in *kanwa* solution; **DAW:** *Daddawa* produced from seeds cooked in pure distilled water*;* **DAD:** *Daddawa* produced from seeds cooked in *dalang* solution; **CC:** cooked cotyledons; **DN:** *Daddawa* bought in Ngaoundere market

Each data point presented as Mean± Standard Deviation (n=3) Means along the same line with different superscript are significantly different at P<0.05.

T**able 1** show that *daddawa* from seeds cooked in *dalang* solution (DAD) has a relatively lower dry matter 89.16±1.90% as compared to distilled water *daddawa* (DAW) *and kanwa daddawa* (DAK) which have 95.3±0.22% and 95.38±0.42% respectively. In other words the dry matter for the DAK is higher by 5.97% to that of the DAD. Statistically, there is significant differences in the dry matters of both *daddawa* condiments produced. Knowing the dry matter of these samples is important because the quality of the samples is best assured when they contain smaller proportions of water. Thus, the DAK can be stored for a longer period of time than DAD in a cool dry place in a tightly closed container because it is quite stable due to the high %DM. However, the water present should not be in the form of free water because it reduces storage stability and consequently the quality of *daddawa.* For precautionary motives, the *daddawa* produced can be further dried.

The close control of the final water content of the *daddawa* condiments produced is therefore important because as little as 1-2% excess water can result in some common defects such as moulding in our final product which will eventually modify the quality of the nutritional contents of the condiments such as the crude protein content.

The protein content of the samples varies according to the treatments. The results in **table 1** show that the highest value is observed in DAK (38.61±0.75% DM) against DAD (35.56±0.47 % DM). From these results, one observes that the mode of fermentation influences the composition of total proteins of *daddawa*. The crude protein content decreases with fermentation. The value drops from 47.01±0.61% DM in CC to 38.61±0.75 % DM, 35.56±0.47 %DM and 34.84±0.67 %DM in DAK, DAD and DAW respectively. These values are lower than those (43.26±0.97%DM) obtained in *daddawa* bought in Ngaoundere market (DN).

According to observations of Doumta and Tchiégang[1]; Rodolfo, Valdez and Mariano[30] protein content of *daddawa* decreases slightly during fermentation. This is in accordance with our results when distilled water and *alkaline* treatments are used in comparison with the values of cooked cotyledons (47.01±0.61% DM).

This slight fall could be due to the weak synthesis of proteins by the micro-organisms which proliferate in the medium, a production of enzymatic proteins or a rearrangement of the other components during the fermentation of cooked cotyledons.[31] In addition, the reduction in proteins would be due to the production of ammonia which generally accompanies fermentation of proteinous foods like *daddawa* and gives it, its characteristic odour.[29] Indeed during fermentation, amino acids release their amino group in the form of ammonia (NH_3) with production of free fatty acids ($R\text{-}CH_2 - COO\text{-}$). The latter will be restructured to give monoglycerides, diglycerides and triglycerides.[2]

The fact that fermentation increases digestibility of proteins could also account for the drop in protein level when compared to the cooked cotyledons. This is because polypeptides chains are broken down by peptidases into smaller chains of amino acids.

The lipid contents vary according to the treatment applied (*kanwa*, *dalang* and distilled water treatments). These contents are defined within the interval 17.35±0.85% DM (CC) to 21.48±0.59% (DAK) and 21.48±0.18% (DAD). These values are in higher than those obtained in **DN** (13.87±0.58% DM). This relatively high lipid level can be due to the carbohydrate metabolism induced by certain micro-organisms transforming the carbohydrates into lipid[32] through the process of transamination decarboxylation with production of free fatty acids.[2] Indeed certain fungi are able to synthesize lipids during fermentation.[33]

The amount of total sugars in the *daddawa* condiments is indicated in **table 1**. These contents vary according to the mode of processing. Thus one passes from 10.67±0.48% DM (CC) to 35.42±0.92% DM (DAK) and 43.39±0.77% DM (DAW). DAW shows the highest amount of total sugars than DAK. Total sugar in DAK is higher than what it is observed in DAD (20.04±0.45%DM). The higher digestibility of carbohydrates and the diffusion of soluble sugars from the seeds and cotyledons during alkaline treatment could explain this difference.

Just like lipids, total sugars increase with fermentation whatever the method of fermentation applied. These results are in contradiction with those obtained by Oboh *et al*.[32] on cotyledons of seeds of *P. biglobosa*. The increase in sugar content can equally be due to the transformation of proteins and lipids to carbohydrates by the process of transamination decarboxylation with production of free fatty acids.[2] These fatty acids are then digested by micro-organisms to produce the sugars used like source of energy, reason why one observes higher values for DAW compared to those of DAK and DAD.

All these compounds (lipids, proteins, carbohydrates) act in synergy to pass from an element to another. The transformation of proteins would thus induce an increase in lipids and carbohydrates. This increase in carbohydrates and in lipids in the fermented products can be a significant source of biochemical energy for the consumers of *Daddawa.*[2]

The energy content of the products which depends on their contents of carbohydrates, protein and fat were ranged from 386.78± 0.75 (CC) to 498. 32±1.11 kcal/100g (DAW). The results indicated that energy value produce by DAK (489.57±1.62 kcal/100g) is higher than DAD (415.72±5.3 kcal/100g). However, the energy value of *daddawa* from Ngaoundere (345.87±0.1 kcal/100g) is lower than the one from DAD. This is due to the higher fat content and higher activity of proteases which can be linked to the amelioration of the production parameters. Doumta and Tchiegang[2] observed that increase production parameters can enrich *daddawa* with protein and fat which explain why the energy content is high. The energy of DAK and DAD are lower than DAW, which indicates that some nutrients were lost during the production process. On the other hand, softening seeds by using alkaline agent speed the softening but also provoke the lost of nutrients. Energy obtained with DAK and DAD are is significantly different at $P<0.05$

Ash content gives an estimate of the amount of minerals contained in a sample. The ash contents presented in table 1 vary from 1.07±0.21 (DAW) to 10.50±0.31% DM (CC). Amount of ash in DAK (2.07±0.14 %DM) is lower than the one found in DAD (5.76±0.25%DM). The value obtained in DAD is not far from DN (6.01±0.98%DM). Ash content of DAK and DAD are significantly different at $P<0.05$. This could be due to the fact that *kanwa* and *dalang* contain much more minerals than distilled water. In the traditional processing of *daddawa* seeds are pound with wood ash which plays the role of abrasive during dehulling thereby imparting its numerous minerals to the **DN** produced thus the ash content is relatively high (6.01±0.98% DM).

II.5. Some mineral elements

Table 2 shows the composition in some minerals of the *daddawa* condiments produced, bought and cooked cotyledons.

The determination of some minerals in the *daddawa* condiments produced was aimed at assessing the influence of the processing technique on the nutritional value of the end products.

Table 2: Composition in some minerals of the *daddawa* condiments used

Element	**CC**	**DAW**	**DAK**	**DAD**	**DN**
Zn	8.08±0.01^{c}	7.07±0.04^{b}	12.91±0.06^{e}	11.05±0.02^{d}	5.25±0.11^{a}

Fe	34.14±0.02[c]	24.13±0.03[b]	116.4±0.16[e]	114.42±0.39[d]	22.63±0.57[a]
K	10.81±0.03[a]	7.77±0.10[a]	11.95±0.15[a]	10.07±0.15[d]	7.80±0.11[a]
Na	24.71±1.04[c]	17.11±0.20[b]	26.21±0.32[d]	23.67±0.65[c]	4.55±0.32[a]
Ca	152.32±1.54[b]	102.26±1.85[a]	474.34±1.44[e]	448.61±1.96[d]	231.67±1.73[c]

DAK: *Daddawa* produced from seeds cooked in *kanwa* solution*;* **DAW:** *Daddawa* produced from seeds cooked in pure distilled water*;* **DAD:** *Daddawa* produced from seeds cooked in *dalang* solution; **DN:** *Daddawa* bought in Ngaoundere.

Each data point presented as Mean± Standard Deviation (n=3) Means along the same line with different superscript are significantly different at P<0.05.

Results in table 2 show that the mineral profile of the various condiments varies significantly according to the mode of fermentation. The analysis of the composition in some minerals of the condiments reveals that calcium (102.26-474.34 mg/100g DM) and iron (22.63-116.4 mg/100g DM) are majors constituents; whereas, zinc (5.25-12.91 mg/100g of DM), potassium (7.77-10.95 mg/100g of DM) and sodium (4.55-26.37 mg/100g DM), are present in average quantities when compared to cooked cotyledons. It is well observed DAK presents the best values interms of minerals composition than DAD. This can be explained by the fact that for softening DAK need a high value (1%W/W) of softening agent than DAD (0.75%W/W). These minerals can diffuse inside the seed and increase the minerals composition.

These results are similar to those obtained by Dashak *et al.*[34]; Osuntogun *et al.*[35] drawn from the literature review on Nigerian *Daddawa* with regard to the zinc content (6.8-22.1 mg/100g DM); iron (12.2 -164.50 mg/100g DM) but with high potassium content (50-1913 mg/100g DM. Differences observed in the values obtained can be due to the nature of the ground, the genetic origin, the geographical source of the seed samples and even due to the method used for the production of the condiment.[1][36] The conditions of production of DAK and DAD would contribute to improve the mineral contents.

The production method of DAK and DAD is antagonistic with that of DAW. Thus, the nutraceutical action of *daddawa* in the treatment of diseases like, anaemia, hypertension, the prevention of cardiovascular diseases could be directed towards a fermentation at room temperature with alkaline treatment. Presence of iron and zinc can improve the immune system by acting as antioxidants. In general observation, mineral elements of DAD are significantly different (P<0.05) to those obtained in DAK

Conclusion

The standardisation of a softening process for African locust bean seeds during the production of *daddawa* has been successfully achieved. The long cooking time (24-72 hours) observed in the traditional process has tremendously been reduced (3 hours) by implementing optimum softening parameters obtained from investigations using *kanwa* and *dalang* as softening agents. The amount 0.75% (*dalang*) and 1% (*kanwa*) and time of introduction of the alkali (60 minutes) have been determined and used in identifying the Specific Softening Time (3 hours) through calculations of PDS. When the seeds are cooked for this Specific Softening Time and then fermented at 25°C for 72 hours following the ameliorated process, the *daddawa* produced shows appreciable physicochemical properties. *Daddawa* produced from *kanwa* treatment of seeds using kanwa is found to be rich in lipids, proteins, carbohydrates and energy while *daddawa* produced from *dalang* treatment is found to be rich in mineral elements and is easily digestible.

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