Impact of sorghum processing on phytate, phenolic compounds and in vitro solubility of iron and zinc in thick porridges

Kayodé, A. P. P., Linnemann, A. R., Nout, M. J. R., & van Boekel, M. A. J. S.

Impact of sorghum processing on phytate, phenolic compounds and *in-vitro* solubility of iron and zinc in thick porridges

Suggested running title: "Antinutrients and Fe and Zn solubility in sorghum porridge "

A. P. P. Kayodé[1], A. R. Linnemann[2] , M. J. R. Nout [2*] & M. A. J. S. van Boekel[2]

[1] Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, 01 BP 526 Cotonou, Bénin

[2] Department of Agrotechnology and Food Sciences, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

* Corresponding author: Fax +31 317 484978

E-mail address: rob.nout@wur.nl

KEYWORDS: sorghum; porridge; milling; sieving; wet cleaning; cooking

Abstract

This study focussed on the impact of process variables on levels of phytate and phenolic compounds, and *in-vitro* solubility of iron (Fe) and zinc (Zn) in sorghum porridges, a major staple in semi-arid tropics. The aim was to identify practices that enhance the mineral availability in this type of staple food. We studied the example of the West African porridge '*dibou*' for which the processing methods involve grain cleaning, milling, sieving and cooking. Regional variations occur in the process, particularly in the cleaning which may be done wet or dry; sieving may be omitted in certain locations. Cleaning reduced the phytate content of the grain by 24-39%, while milling, sieving and cooking had no significant effect on phytate. Phenolic compounds measured as levels of reactive hydroxyl groups, remained constant after cleaning, milling and sieving, but significantly decreased by 38-65 % after cooking. The Fe solubility tended to increase after cleaning but was drastically reduced due to cooking, and so was the soluble Zn. Levels of total phenolic compounds highly correlated with the Fe and Zn solubility with a r^2 of 0.73 and 0.82, respectively. Phenolic reaction products formed during the cooking process are presumably related with the extensive browning phenomenon observed in the *dibou* porridge, and with the reduction observed in Fe and Zn solubility.

Introduction

Iron (Fe) and zinc (Zn) are essential trace elements for human nutrition. They support important functions in the organism; their deficiencies in the diet lead to much suffering; particularly in developing countries where cereals and vegetables are the main sources of macro- and micronutrients for the population [1,2]. The mineral content and bioavailability in cereals like sorghum are low due to the presence of anti-nutritional factors such as condensed phenolic compounds and phytate. These form insoluble complexes with essential minerals such as calcium, iron and zinc at physiological pH levels rendering them unavailable for the organism [2,3].

Sorghum [*Sorghum bicolor* (L.) Moench] is an important staple food in semi-arid regions worldwide [4,5]. The grain is processed into various foods including thin or thick porridges and beverages. Porridges reportedly are most commonly prepared from sorghum [4]. *Dibou*, a thick sorghum porridge from Benin is also popular in other countries in the West Africa region. It is known as *tô* in Burkina-Faso and *oka-baba* in Nigeria. It is consumed during lunch or dinner as a main dish, with okra (*Abelmoschus esculentus*), or vegetable soup with meat or fish, depending on the household budget [6]. In spite of their high frequency of consumption among the sorghum foods, little is known about the micronutrient availability from sorghum porridges.

Basically, the preparation of *dibou* involves cleaning of sorghum grain, grinding and cooking with variations according to regional traditions. Cleaning may be done simply by dry sorting and winnowing, or wet by washing in water. Likewise, sieving is an optional operation, which may be systematically omitted from, or included in the process [6]. Also the cooking time may vary depending on the operators. The impact of these process operations on the levels of micronutrients and their availability in porridge is not yet known, nor understood.

In cereal processing, wet cleaning, grinding and sieving serve to remove debris, germs and bran from the grain. In roller milling of *e.g.* wheat, wet cleaning induces water uptake of the pericarp, which enhances its flexibility and resistance to friction during milling, permitting its separation from the endosperm in the form of large flakes [7,8,9]. Subsequent sieving therefore efficiently removes bran. Anti-nutritional factors, such as tannins and phytates, are mainly concentrated in the bran and the aleuronic layer of the grain [2,10]. Against this background and assuming some similarity of roller milling and disc attrition milling such as practiced in village-style sorghum processing, it is hypothesized that *dibou* from sorghum that is washed or/and sieved during processing, contains lower levels of anti-nutritional factors and has higher solubility of Fe and Zn. No studies were published on the impact of household processing methods on anti-nutritional factors, or Fe and Zn solubility in sorghum porridge. Contradicting information exists on the impact of cooking on phytate content of food crops. Fretzdroff and Weiper [11] reported that cooking at 100 °C did not affect phytate content of rye flour. Similarly, no reduction in phytate was observed when yam flour was cooked [12]. But instead, a decrease in phytate content of sorghum and pigeon pea (*Cajanus cajan*) was observed when the milled grain was cooked [13,14].

The present study investigated the current household sorghum processing methods to prepare *dibou* in two communities in the Benin sahelian zone. We focus on the impact of process operations on phytate, phenolic compounds and Fe and Zn content, aiming to identify the household practices that enhance the level of Fe and Zn solubility in the porridge.

Materials and methods

Household survey

Fifty-two households, previously identified as *dibou* consumers, were surveyed in two regions (*Parakou* and *Natitingou)* of northern Benin. These regions had been selected on the basis of their socio-cultural diversity. Households were chosen randomly and differed from each other in terms of their socio-cultural background. The respondents were the housewives who take care of food preparation for the family. The questionnaire included the following aspects: the sorghum varieties used and quantity processed, the unit operations involved in making *dibou*, and the quantification of equipments, time and fuel. Occasionally, housewives were closely observed while carrying out the preparation. The protocol used for the survey was approved by the Faculty of Agronomical Sciences of the University of Abomey-Calavi; informed consent was obtained from all participating households.

Processing and sampling

One batch of red sorghum [*Sorghum bicolor* (L.) Moench] was purchased at a local market in *Parakou* and processed into *dibou* following three representative process scenarios resulting from the survey (Figure 1). Five kg of grain were processed into *dibou* by duplicate households for each process scenario. Samples –sorghum grain, semi-processed grain and porridges- were withdrawn at each process step, dried in an oven, ground into flour using a *Retsch* mill (Retsch bv, type ZM 1) fitted with a 0.5 mm screen and stored at –20°C until analysis.

In-vitro digestion of samples for analysis of soluble Fe and Zn

The *in-vitro* digestion method [15] was used, with minor modifications. Duplicate dry samples of flour (5 g) were suspended in 30 ml distilled water and digested under simulated gastrointestinal conditions, using α-amylase solution (Sigma A-1031), stomach medium consisting of lipase (Amano Pharmaceuticals, Rhizopus F-AP15) and pepsin (Sigma P-6887), and pancreatic solution consisting of pancreatin (Sigma P-1750) and bile (Sigma B-3883). After digestion, the suspension was centrifuged at 3600 g for 15 min at 4°C. The supernatant was decanted and the pellet was washed twice in 20 ml of distilled water and centrifuged. The supernatants were pooled and filtered through a 0.45 µm pore filter. A blank was included consisting of 30 ml distilled water digested and filtered as described above. Both filtered supernatants from sample and blank were analysed for Fe and Zn. Samples were corrected for added reagents/water by subtracting Fe and Zn content of blank from that of supernatants from samples. The amounts of Fe and Zn (expressed per mg/kg of digested sample) in supernatant were regarded as soluble minerals. Percentage of soluble mineral was calculated as:

Solubility (%) = {(Fe or Zn in supernatant – Fe or Zn in blank) / (Fe or Zn in undigested sample)} x 100.

Physico-chemical analysis

Fe and Zn determination

Approximately 0.4 g of sorghum flour was digested using hydrofluoric acid (40%) and concentrated nitric acid (65 % w/w). Next, the concentrations of Fe and Zn were analysed by the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Elan 6000, Perkin Elmer, USA) [16]. Samples from *in-vitro* digestion were collected in tubes (10 ml) and 0.15 ml of concentrated nitric acid (HNO_3 65%) was added to preserve them. These samples were analysed by the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, Elan 6000, Perkin Elmer, USA). Measurements were performed in duplicate.

Phytate determination

Approximately 10 mg of grain flour was extracted with 1 ml of 0.5N HCl containing 50 mg/l cis-aconitate (internal standard) [17]. The mixture was boiled in a water bath at 100°C for 15 min and then centrifuged at 14,000 g for 10 min. The supernatant was diluted 5x in millipore water and analysed using HPLC (Dionex DX300, ICS2500 system, detector range of 10 µS) using the column AS11 (ATC column + guard column). Detection was with suppressed conductivity and the suppression was done with water at a flow rate of 5 ml/min. The eluent and the elution times used are as follows: 0-5 min 5 mM NaOH ; 5-15 min 5-100 mM NaOH ; 15-20 min 500 mM NaOH and 20-35 min 5 mM NaOH. A standard solution was prepared in millipore water, that contains 5.0 mg/l $NaNO_3$ (Merck p.a.), 5.0 mg/l Na_2SO_4, (Merck p.a.), 5.0 mg/l Oxalic acid.$2H_2O$ (Merck p.a.), 10.0 mg/l $Na_2HPO_4.2H_2O$ (Merck 6346 p.a.), 10 mg/l citric acid, H_2O (Merck K23524044 719 p.a.), 5.0 mg/l cis-aconitate (Aldrich 27194-2) and 10 mg/l $IP_6.Na_{12}$ (Sigma P3168 lot 102K0053). Measurements were performed in triplicate.

Total phenolics determination

Total phenolic compounds (PC) were extracted from 50 mg of flour in 1.5 ml of HCl/methanol (1% v/v) for 1 h under continuous stirring at room temperature. The mixture was centrifuged at 5,000 g for 10 min and supernatant was removed. Next the pellet was re-extracted as described above and supernatants were pooled [18]. The PC were measured following the method of Singleton and Rossi [19] modified as follows. 300 µl of extract were added with 4.2 ml of distilled water, 0.75 ml of Folin-Ciocalteau's reagent (Merck, Germany) and 0.75 ml of sodium carbonate solution (20% w/v). After incubation for 30 min the optical density was measured at 760 nm using a spectrophotometer (*Shimadzu* UV 240, Kyoto, Japan). Blanks were always freshly prepared, in which Folin-Ciocalteau's reagent was replaced by water to correct for interfering compounds. Gallic acid (New Jersey, USA) was used as standard and the results were expressed as gallic acid equivalent per g of samples.

Crude protein, ash and colour measurement

Crude protein (N x 6.25) and ash were determined according to the AOAC method [20]. The colour of grain samples was measured with a Minolta CR-210 portable chromameter (Illuminant D65 CIE 1976) standardized with a standard white tile (Y = 94.8, x = 0.315 and y = 0.3324). The L, a*, b* values were recorded (L= whitness index, a*= redness index , b*= yellowness index) and the browning index was calculated as: BI = 100-L [21]. Because adding water to flour may lead to colour changes as observed in our own experiments (data not shown), we took this into account in the interpretation of data on cooked flour.

Statistical analysis

Survey data were analysed using *Winstat 2.0* software. For the analytical data, mean values and standard deviation are reported. The data were analysed using the statistical program SPSS 11.0 and the one-way ANOVA model was used applying the LSD test to evaluate significant difference among means.

Results and discussion

Variation in household dibou processing

The unit operations involved in *dibou* preparation and the percentage of households using them are presented in Table 1. In general, *dibou* preparation involves cleaning, grinding, sieving and cooking. Cleaning may consist exclusively of a simple sorting and winnowing of grains (70% of households), or washing in water (30%). The proportion of households using dry or wet cleaning methods depends on the region. In *Natitingou*, most of the processors sort the grain, while in *Parakou*, half of the households use sorting while the other half wash to clean the grains. Sieving is not used by 40 %; most households that sieve the flour are located in *Parakou*. These process variations lead to three scenarios of *dibou* preparation as shown in Figure 1. The interviewed housewives explained that the main reasons for washing the grain or sieving the flour, are to improve the palatability and to enhance the textural properties (particularly the elasticity) of the final product. Most processors in *Natitingou* perceived washing and sieving as time-consuming tasks, which explains the low proportion of households using these operations there. Indeed, washing necessitates a drying step, which takes 1-2 hours depending on solar intensity. Processors in Natitingou sometimes add cassava chips to the grain to obtain the desired texture (elasticity) in the paste; we did not take this addition into account in the comparison of processing scenarios. In the following sections the nutritional impacts of the different scenarios are discussed.

Impact on total Fe and Zn content

The variations in Fe and Zn content of sorghum grain during *dibou* preparation following the three process scenarios are presented in Table 2. The Zn content of the grain remains constant throughout the process with a slight increase after cooking, possibly due to contamination from the metallic cooking pot. The Fe and ash (in scenarios 2 and 3) content also increased after cooking. The washing process (scenario 3) significantly reduces the grain-Fe by 67%. The mineral balance (Table 3) also reveals a significant loss in Fe after the washing process in scenario 3 Indeed, the Fe content of the grain (256 mg/kg) found in this study is high when compared to earlier values reported for sorghum seed. Kayodé *et al.* [22] reported a mean value of 57.5 mg/kg with a range of 32-99 in 45 sorghum genotypes from Northern Benin. Jambunathan [23] reported an average Fe content of 59 mg/kg with a range of 26-96 mg/kg in samples of about 100 varieties of sorghum. The origin of our grain, which was bought at local market, may be responsible for this discrepancy. The grain may have been contaminated during post-harvest treatments, notably during the threshing, which consist of beating the ears on the ferruginous soil. The fact that the Fe content of the grain was drastically reduced after washing (scenario 3) supports this hypothesis. Unexpectedly, sieving did not affect the mineral content of the flour. This can be explained by the fact that grinding reduced the grain into fine powder and subsequent sieving did not result in the selective separation of *e.g.* testa. The analysis of mass balances (Table 3) showed a slight loss of coarse material due to sieving.

Impact on phytate and total phenolics

Table 4 shows a grain-phytate level of 0.8 %; this is in agreement with earlier findings [24,25]. As can be seen in Table 4, cleaning reduces the phytate content of the grain by 24-25% after dry cleaning (Scenarios 1 and 2), and by 39% after wet cleaning (Scenario 3), respectively. The decrease from cleaning is greater than achieved by soaking, where 16-21% phytate reduction was reported [13], but is similar to decreases caused by germination [24]. Thus, cleaning can significantly contribute to phytate removal from sorghum-based foods. The decreased phytate content may be due to removal of exogenous materials such as grains with attached glumes, spoiled grains, and attrition dust. The wet cleaning appeared to be more efficient in removing these exogenous particles.

Cooking did not affect the phytate content, in contrast to another observation of decreased phytate content of sorghum flour after cooking [13]. Our results resembled findings for yam and rye flour, in which phytate was reported to be stable under the ordinary wet cooking conditions [11,12]. The total phenolic compounds measured by their reactive hydroxyl groups, significantly decreased during cooking in all process scenarios (Table 2), the decrease ranging from 38 to 65 %. During heating, the phenolic hydroxyl groups may have reacted, or formed insoluble complexes with food components such as protein and minerals, or even polymerized into condensed phenolics leading to a decrease of assayable phenolic hydroxylic groups [26,27,28].

Impact on in-vitro solubility of Fe and Zn

The levels of soluble Fe and Zn at each process step are presented in Table 2. In the final product (*dibou*) the level of soluble Fe ranged from 6.2 to 13.3 mg/kg with an average of 9.4 mg/kg (dry basis). Values for soluble Zn ranged from 1.9 to 3.4 mg/kg with an average of 2.5 mg/kg. In all scenarios the *in-vitro* soluble Fe increased significantly after cleaning and remained quite constant after grinding and sieving. This trend seems to follow the changes in phytate content, which decreased after cleaning and remained constant after grinding and sieving (see above). The *myo*-inositol hexakisphosphate (IP6) is the major inhibitor of Fe and Zn absorption from plant foods, and lowering the levels of phytic acid in meals of plant origin could greatly improve the absorption of these minerals [2]. Contrary to our expectation, no correlation could be established between Zn solubility and the phytate content of the flours. Possibly, this is related to the fact that Fe and Zn are not located in the same place in the seed.

Zn is found in a large number of enzymes and other proteins and is distributed throughout the seed [29]. Fe in seeds is stored as phytoferritin or phytate, mainly concentrated in the bran and the aleuronic layer of the grain [10,2].

During the three process scenarios studied, cooking drastically reduced the *in-vitro* Fe and Zn solubility in the porridge. This reduction could not be linked to the inhibitory effect of phytate, which remained constant after cooking. After cooking, a 56-68% reduction in soluble Fe occurred and the solubility in Zn was reduced by 57-76%. Matuscheck *et al.* [28] also reported a significant decrease of *in-vitro* soluble Fe after cooking sorghum flour and related this to the chelating effect of phytate and phenolic compounds. Phenolic compounds, especially condensed phenolics such as tannins, are also reported to chelate divalent minerals *i.e.* Fe and Zn [2]. Our results indicate significant positive correlations ($P < 0.01$) between the level of reactive phenolic hydroxyl groups and the Fe and Zn solubility (Table 5). During heat treatments, *e.g.* cooking, the phenolic compounds can polymerise into condensed phenolics leading to a decrease of the assayable total phenolics. Hence in this study, we suspected the condensed phenolics to be responsible for the considerable decrease of soluble Fe and Zn observed after cooking. The extensive browning of the flour observed after cooking (Table 6) and the colouration behaviour associated with condensed phenolic compounds [3,2] would support this hypothesis.

Conclusion

The present study evaluated the impact of process unit operations used to prepare sorghum thick porridge (*dibou*) at the poorest household level in Benin, on the *in-vitro* solubility of micronutrients. Cleaning, especially wet cleaning, significantly contributes to phytate removal from sorghum grain and results in better Fe solubility. Sieving of milled grain as currently applied, is less effective in achieving reduction of phytate and phenolic contents of the grain flour. Sieving might be more efficient if grains are first conditioned by moistening and then coarsely ground, prior to sieving. Cooking was found to be the main unit operation that restricts the Fe and Zn availability in porridge. Further research is recommended to identify the inhibitors of mineral solubility generated during cooking, and to develop approaches that alleviate the chelating effects.

Aknowledgement

Financial support provided by Wageningen University through the North-South Interdisciplinary Research and Education Fund (INREF) is gratefully acknowledged. The International Foundation for Science, Stockholm, Sweden is acknowledged for financial support to A. P. P. Kayodé (Grant N° IFS E/3736-1).

References

1. Svanberg U and Lorri W, Fermentation and nutrient availability. *Food Control* **8**:319-327 (1997).
2. Frossard E, Bucher M, Mächler F, Mozafar A and Hurrell R, Potential for increasing the content and bioavailability of Fe, Zn, and Ca in plants for human nutrition. *Journal of the Science of Food and Agriciculture* **80**:861-879 (2000).
3. Graf E, Chemistry and application of phytic acid: An overview. In Graf E.(ed.) Phytic acid: Chemistry and application. *Pilatus Press*: Minneapolis, MN, pp 1-21 (1986).
4. Duodu KG, Taylor JRN, Belton PS, and Hamaker BR, Factors affecting sorghum protein digestibility. *Journal of Cereal Sci*ence **38**:117-131 (2003).
5. Hounhouigan DJ, La valorisation des céréales locales pour les marchés urbains en Afriques de l'Ouest: les atouts, contraintes et perspectives, in *Food based approaches for a healthy nutrition in West Africa; Proceedings of the 2nd international workshop*, Brouwer I.D., Traore A.S. and Treche S. University of Ouagadougou, Ouagadougou; Institute for Development Research, Montpellier; Wageningen University, Wageningen; Food and Agriculture Organization, Rome, Ouagadougou, pp 359-368 (2004).
6. Kayodé APP, Adégbidi A, Linnemann AR, Nout MJR and Hounhouigan DJ, Quality of farmer's varieties of sorghum and derived foods as perceived by consumers in Benin. *Ecology of Food and Nutrition* **44**:271-294 (2005).
7. Brekke O L and Kwolek WF, Corn dry-milling: cold-tempering and degermination of corn of various initial moisture content. *Cereal Chemistry* **46**:545-559 (1969).
8. Reichert RD, Sorghum dry milling. In sorghum in the eighties : *Proceedings of the international symposium on sorghum, 2-7 November 1981, Patancheru, AP, India (Vol.2). ICRISAT, Patancheru, AP, India. pp.* 547-564 (1982).
9. Sahay KM, Evaluation of a general purpose abrasive mill for pearling of coarse cereals and dehusking of pulses. *International Journal of Food Science and Technology* **25**:220-225 (1990).
10. Beta T, Rooney LW, Marovatsanga L and Taylor JRN, Phenolic compounds and kernel characteristics of Zimbabwean sorghums *Journal of the Science of Food and Agriculture* **79**:1003-1010 (1999).
11. Fretzdroff B and Weiper D, Phytic acid in cereals: phytase in rye and rye products. *Zeitschrilt fur Lebensmittel Untersuchung und –Forschung* **82**:287-293 (1986).
12. Wanasundera JPD and Ravindran G, Effect of cooking on the nutrient and antinutrient contents of yam tubers (*Dioscorea alata* and *Dioscorea esculenta*). *Food Chemistry* **45**:247-250 (1992).
13. Mahgoub SEO and Elhag SA, Effect of milling, soaking, malting, heat-treatment and fermentation on phytate level of four Sudanese sorghum cultivars. *Food Chemistry* **61**:77-80 (1998).
14. Duhan A, Khetarpaul N and Bishnoi S, Content of phytic acid and HCl-extractability of calcium, phosphorus and iron as affected by various domestic processing and cooking methods. *Food Chemistry* **78**:9-14 (2002).
15. Kiers JL, Nout MJR and Rombouts FM, In vitro digestibility of processed and fermented soya bean, cowpea and maize. *Journal of the Science of Food and Agriculture* **80**:1325-1331 (2000).
16. Temminghof, E. (2000). Soil and Plant Analysis. Part 3. Plant analysis procedures. Wageningen University Environmental Sciences, Wageningen (1997).
17. Bentsink L, Yuan K, Koornneef M and Vreugdenhil D, The genetics of phytate and phosphate accumulation in seeds and leaves of *Arabidopsis thaliana*, using natural variation. *Theoretical and Applied Genetics* **106**: 1234-1243 (2003).
18. Cia T, Ejeta G and Butler L G, Screening for grain polyphenol variants from high-tanin sorghum somaclones. *Theoretical and Applied Genetics* **90**:211-220 (1995).
19. Singleton VL and Rossi JA, Colorimetry of total phenolic compounds with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**:144-158 (1965).

20. American Association of Cereal Chemists, Approved Method of the AACC, *St Paul Minnesota 8th edition* (1984).
21. Mestres C, Dorthe S, Akissoe N and Hounhouigan DJ, Prediction of sensorial properties (colour and taste) of amala, a paste from yam flour of West Africa, through flour biochemical properties. *Plant Foods for Human Nutrition* **59**:93-99 (2004).
22. Kayodé APP, Linnemann AR, Hounhouigan DJ, Nout MJR and van Boekel MAJS, Genetic and environmental impact on iron, zinc and phytate in food sorghum grown in Benin (submitted).
23. Jambunathan R, Improvement of the nutritional quality of sorghum and pearl millet. *Food and Nutrition Bulletin* **2**:11-16 (1980).
24. Traoré T, Mouquet C, Icard-Vernière C, Traoré AS and Trèche S, Changes in nutrient composition, phytate and cyanide contents and α-amylase activity during cereal malting in small production units in Ouagadougou (Burkina Faso). *Food Chemistry* **88**:105-114 (2005).
25. Reddy NR, Occurrence, distribution, content, and dietary intake of phytate. In N.R. Reddy and S. K. Sathe (eds.), Food phytates (pp. 25-52). Boca Raton: CRC Press (2002).
26. Barroga CF, Laurena AC and Mendoza EMT. Polyphenols in mung bean (*Vigna radiata* (L.) Wilczek): determination and removal. *Journal of Agricultural and Food Chemistry* **33**:1006-1009 (1985).
27. Ekpenyong TE, Effect of cooking on polyphenolics content of some Nigerian legumes and cereals. *Nutrition Reports International* **31**:561-565 (1985).
28. Matuscheck E, Towo E and Svanberg U, Oxidation of polyphenols in phytate-reduced high-tannin cereals: *Effect* on different phenolic groups and on in vitro accessible Iron. *Journal of Agricultural and Food Chemistry* **49**:5630-5638 (2001).
29. Lestienne I, Icard-Vernière C, Mouquet C, Picq C and Trèche S, Effect of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chemistry* **89**:421-425 (2005).

Table 1 : Frequency of use of unit process operations involved in *dibou* preparation by 52 households from 2 regional communities in Northern Benin (in % of n respondents)

	Region		
Unit operations	*Parakou* (n=30)	*Natitingou* (n=22)	Total (n=52)
Sorting	52	95	70
Washing	48	5	29
Drying	48	5	29
Grinding	100	100	100
Sieving	90	18	60
Cooking	100	100	100

Table 2: Changes in iron, zinc, ash, crude protein and phenolics content of sorghum grain during *dibou* preparation

	Total Fe[1] (mg kg^{-1} dm)	IVS[2] Fe (mg kg^{-1} dm)	Total Zn (mg kg^{-1} dm)	IVS Zn (mg kg^{-1} dm)	Ash (g 100 g^{-1} dm)	Crude protein (g 100 g^{-1} dm)	Total phenolics (g 100 g^{-1} dm)
Scenario 1 *(dry cleaning - grinding - cooking)*							
raw	255.8±25.2a	15.1±0.6a	25.4±0.5a	8.4±0.6a	1.8±0.0a	10.5±0.1a	0.22±0.00a
cleaned	177.8±38.3a	34.9±7.5b	25.7±0.7a	7.9±0.3a	1.8±0.1a	10.2±0.2a	0.26±0.01a
ground	178.5±37.5a	34.0±0.7b	24.5±0.3a	7.3±0.8a	1.8±0.0a	10.1±0.1a	0.26±0.01a
cooked	212.7±43.6a	13.2±1.7a	27.0±1.5a	3.4±3.7b	1.8±0.3a	10.1±0.2a	0.16±0.02b
Scenario 2 *(dry cleaning - grinding - sieving - cooking)*							
raw	255.8±25.2a	15.1±0.6a	25.4±0.5a	8.4±0.6a	1.8±0.0a	10.5±0.1a	0.22±0.00a
cleaned	304.1±64.5a	25.3±2.0b	27.2±1.4a	9.2±0.3a	1.9±0.3b	9.7±0.2b	0.23±0.02a
ground	310.4±57.4a	25.8±1.2b	26.8±1.3a	9.4±0.7a	1.8±0.2b	9.7±0.1b	0.23±0.00a
sieved	277.7±31.0a	26.5±0.3 b	25.7±0.8a	8.7±0.1 a	1.9±0.1b	9.7±0.3b	0.23±0.02a
cooked	314.1±36.7a	8.6±1.8c	30.5±6.3a	2.1±1.1b	2.0±0.2b	10.7±0.4a	0.08±0.02b
Scenario 3 *(wet cleaning - grinding - sieving - cooking)*							
raw	255.8±25.2a	15.1±0.6a	25.4±0.5a	8.4±0.6a	1.8±0.0a	10.5±0.1a	0.22±0.00a
cleaned	70.7±3.1b	14.8±0.9a	26.2±1.6a	6.0±1.3b	1.7±0.1b	9.8±0.6a	0.24±0.01a
ground	73.1±7.4b	14.1±0.5a	27.2±0.9a	5.4±1.1b	1.7±0.1b	9.7±02a	0.24±0.01a
sieved	69.2±0.4b	14.3±0.7a	26.0±0.9a	5.8±0.7b	1.7±0.4b	9.8±0.3a	0.23±0.02a
cooked	99.4±0.6c	6.3±0.6b	28.1±0.1c	1.9±0.0c	2.1±0.1c	9.9±0.8a	0.15±0.04b

[1] Means ± standard deviation, means with the same letter are not significantly different according to the LSD at the 0.05 level; [2] IVS: *in-vitro* soluble

Table 3: Balances of mass[1], Fe and Zn during *dibou* preparation

	Mass (kg dm) [2]	Fe (g dm)	Zn (g dm)
Scenario 1 *(dry cleaning - grinding - cooking)*			
raw	100±0.0a	25.6±2.5a	2.5±0.1a
cleaned	94.8±0.3b	16.8±2.9a	2.4±0.6ab
ground	87.5±2.0c	15.5±2.4b	2.3±0.9b
cooked	84.5±0.2d	18.0±4.4a	2.3±0.9b
Scenario 2 *(dry cleaning - grinding - sieving - cooking)*			
raw	100±0.0a	25.6±2.5a	2.5±0.1a
cleaned	96.2±1.7b	29.2±6.7a	2.6±0.1a
ground	92.5±0.5bc	28.1±7.0a	2.5±0.3a
sieved	90.3±1.3c	25.1±3.6a	2.3±0.3a
cooked	82.5±2.5d	26.0±4.4a	2.5±0.4a
Scenario 3 *(wet cleaning - grinding - sieving - cooking)*			
raw	100±0.0a	25.6±2.5a	2.5±0.1a
cleaned	95.5±2.7ab	6.8±0.4b	2.5±0.2a
ground	89.4±6.1bc	6.3±0.6b	2.3±0.3a
sieved	85.3±4.6c	5.9±0.3b	2.2±0.2a
cooked	79.9±1.3c	7.9±0.1b	2.2±0.2a

[1] The quantity of product obtained at each process step was carefully weighed during *dibou* processing, using a scale. The generated values were combined with data on dry matter, Fe and Zn concentrations of the different products, to calculate the data presented in this table.

[2] Means ± standard deviation, means with the same letter are not significantly different according to the LSD at the 0.05 level

Table 4: Changes in phytate (IP6) and in-vitro soluble ratio iron and zinc in sorghum grain during *dibou* preparation

	IP6 ($g\ 100\ g^{-1}$ dm) [1]	IVS Ratio Fe[2]	IVS Ratio Zn[3]
Scenario 1 *(dry cleaning - grinding - cooking)*			
raw	0.80±0.13a	5.9±0.3a	33.3±2.4a
cleaned	0.61±0.12b	19.9±3.7b	30.6±1.9a
ground	0.61±0.12b	20.0±0.5b	29.9±1.3a
cooked	0.70±0.06b	6.3±0.6a	5.6±3.0b
Scenario 2 *(dry cleaning - grinding - sieving - cooking)*			
raw	0.80±0.13a	5.9±0.3a	33.3±2.4a
cleaned	0.60±0.01b	8.7±2.6b	33.9±1.3a
ground	0.60±0.01b	8.9±2.5b	33.6±1.1a
sieved	0.59±0.04b	9.6±1.2b	33.7±1.0a
cooked	0.62±0.06b	2.8±0.9c	7.3±4.5b
Scenario 3 *(wet cleaning - grinding - sieving - cooking)*			
raw	0.80±0.13a	5.9±0.3a	33.3±2.4a
cleaned	0.49±0.07b	20.9±1.4b	22.6±3.9b
ground	0.49±0.07b	20.9±1.3b	22.1±2.9b
sieved	0.51±0.09b	20.6±1.2b	22.1±2.1b
cooked	0.51±0.16b	6.3±0.6c	7.0±0.0c

[1] Means ± standard deviation, means with the same letter are not significantly different according to the LSD at the 0.05 level; [2] In Vitro Soluble Ratio Fe = [{IVS Fe ($mg\ kg^{-1}$ dm)} / {Total Fe ($mg\ kg^{-1}$ dm)}] x 100; [3] In Vitro Soluble Ratio Zn = [{IVS Zn ($mg\ kg^{-1}$ dm)} / {Total Zn ($mg\ kg^{-1}$ dm)}] x 100.

Table 5: Pearson correlation matrix between IVS Fe, IVS Zn, Phytate (IP6), reactive phenolic hydroxyl groups and the browning index of sorghum

	IVS Fe	IVS Zn	IP6	PC
IVS [1] Zn	0.359			
IP6	0.398	-0.477		
PC[2]	0.729*	0.823**	-0.339	
BI[3]	-0.667*	-0.912**	0.580	-0.921**

** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level; [1] IVS: *in-vitro* soluble; [2] PC : total phenolic compounds; [3]BI : browning index (BI = 100- L, L is the whiteness index).

Table 6: Colour changes of sorghum during *dibou* preparation

	Browning index (BI = 100-L)		
	Scenario 1	Scenario 2	Scenario 3
raw	23.6±0.7a[1]	23.6±0.7a	23.6±0.7a
cleaned	23.6±0.7a	23.7±0.2a	22.2±0.1a
ground	23.6±0.6a	23.7±0.2a	22.2±0.1a
sieved	-	23.7±0.3a	22.2±0.2a
cooked	49.3±1.3b	50.3±1.2b	46.6±0.2b

[1] Means ± standard deviation, means with the same letter are not significantly different according to the LSD at the 0.05 level.

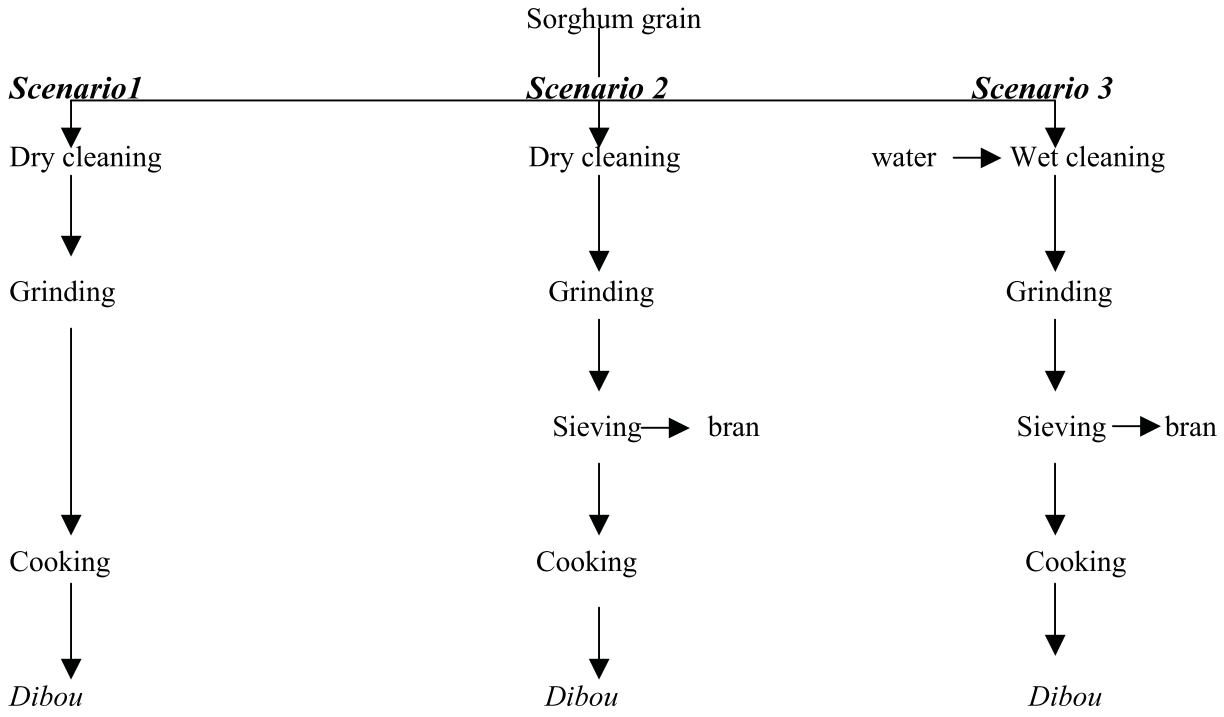

Figure 1: Process diagrams showing the 3 scenarios of *dibou* production