

This is a "Post-Print" accepted manuscript, which has been published in the "Journal of the Science of Food and Agriculture".

Please cite this publication as follows:

Kayode, A.P.P., Linnemann, A.R., Nout, M.J.R. and Van Boekel, M. (2007) Impact of sorghum processing on phytate, phenolic compounds and in vitro solubility of iron and zinc in thick porridges. *Journal of the Science of Food and Agriculture* 87, 832-838.

You can download the published version at:

<http://dx.doi.org/10.1002/jsfa.2782>

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

3
4
5
6
7 Suggested running title: “Antinutrients and Fe and Zn solubility in sorghum porridge ”

8
9
10
11 **A. P. P. Kayodé¹, A. R. Linnemann², M. J. R. Nout^{2*} & M. A. J. S. van Boekel²**

12
13
14
15 ¹ Faculté des Sciences Agronomiques, Université d’Abomey-Calavi, 01 BP 526 Cotonou,
16 Bénin

17 ² Department of Agrotechnology and Food Sciences, Wageningen University, P.O. Box 8129,
18 6700 EV Wageningen, The Netherlands

19
20
21
22
23
24
25
26
27
28
29 * Corresponding author: Fax +31 317 484978

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

31
32
33
34
35
36
37
38 **KEYWORDS:** sorghum; porridge; milling; sieving; wet cleaning; cooking

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

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.

1 **Introduction**

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

11
12 Sorghum [*Sorghum bicolor* (L.) Moench] is an important staple food in semi-arid
13 regions worldwide ^{4,5}. The grain is processed into various foods including thin or thick
14 porridges and beverages. Porridges reportedly are most commonly prepared from sorghum ⁴.
15 *Dibou*, a thick sorghum porridge from Benin is also popular in other countries in the West
16 Africa region. It is known as *tô* in Burkina-Faso and *oka-baba* in Nigeria. It is consumed
17 during lunch or dinner as a main dish, with okra (*Abelmoschus esculentus*), or vegetable soup
18 with meat or fish, depending on the household budget ⁶. In spite of their high frequency of
19 consumption among the sorghum foods, little is known about the micronutrient availability
20 from sorghum porridges.

21
22 Basically, the preparation of *dibou* involves cleaning of sorghum grain, grinding and
23 cooking with variations according to regional traditions. Cleaning may be done simply by dry
24 sorting and winnowing, or wet by washing in water. Likewise, sieving is an optional
25 operation, which may be systematically omitted from, or included in the process ⁶. Also the
26 cooking time may vary depending on the operators. The impact of these process operations on

1 the levels of micronutrients and their availability in porridge is not yet known, nor
2 understood.

3
4 In cereal processing, wet cleaning, grinding and sieving serve to remove debris, germs
5 and bran from the grain. In roller milling of *e.g.* wheat, wet cleaning induces water uptake of
6 the pericarp, which enhances its flexibility and resistance to friction during milling, permitting
7 its separation from the endosperm in the form of large flakes ^{7,8,9}. Subsequent sieving
8 therefore efficiently removes bran. Anti-nutritional factors, such as tannins and phytates, are
9 mainly concentrated in the bran and the aleuronic layer of the grain ^{2,10}. Against this
10 background and assuming some similarity of roller milling and disc attrition milling such as
11 practiced in village-style sorghum processing, it is hypothesized that *dibou* from sorghum that
12 is washed or/and sieved during processing, contains lower levels of anti-nutritional factors
13 and has higher solubility of Fe and Zn. No studies were published on the impact of household
14 processing methods on anti-nutritional factors, or Fe and Zn solubility in sorghum porridge.
15 Contradicting information exists on the impact of cooking on phytate content of food crops.
16 Fretzdorff and Weiper ¹¹ reported that cooking at 100 °C did not affect phytate content of rye
17 flour. Similarly, no reduction in phytate was observed when yam flour was cooked ¹². But
18 instead, a decrease in phytate content of sorghum and pigeon pea (*Cajanus cajan*) was
19 observed when the milled grain was cooked ^{13,14}.

20
21 The present study investigated the current household sorghum processing methods to
22 prepare *dibou* in two communities in the Benin sahelian zone. We focus on the impact of
23 process operations on phytate, phenolic compounds and Fe and Zn content, aiming to identify
24 the household practices that enhance the level of Fe and Zn solubility in the porridge.

25
26

1 **Materials and methods**

2

3 *Household survey*

4

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

15

16 *Processing and sampling*

17

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

25

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

27 The *in-vitro* digestion method ¹⁵ was used, with minor modifications. Duplicate dry samples
28 of flour (5 g) were suspended in 30 ml distilled water and digested under simulated gastro-

1 intestinal conditions, using α -amylase solution (Sigma A-1031), stomach medium consisting
2 of lipase (Amano Pharmaceuticals, Rhizopus F-AP15) and pepsin (Sigma P-6887), and
3 pancreatic solution consisting of pancreatin (Sigma P-1750) and bile (Sigma B-3883). After
4 digestion, the suspension was centrifuged at 3600 g for 15 min at 4°C. The supernatant was
5 decanted and the pellet was washed twice in 20 ml of distilled water and centrifuged. The
6 supernatants were pooled and filtered through a 0.45 μ m pore filter. A blank was included
7 consisting of 30 ml distilled water digested and filtered as described above. Both filtered
8 supernatants from sample and blank were analysed for Fe and Zn. Samples were corrected for
9 added reagents/water by subtracting Fe and Zn content of blank from that of supernatants
10 from samples. The amounts of Fe and Zn (expressed per mg/kg of digested sample) in
11 supernatant were regarded as soluble minerals. Percentage of soluble mineral was calculated
12 as:

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

15 *Physico-chemical analysis*

16 *Fe and Zn determination*

17
18
19
20 Approximately 0.4 g of sorghum flour was digested using hydrofluoric acid (40%) and
21 concentrated nitric acid (65 % w/w). Next, the concentrations of Fe and Zn were analysed by
22 the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Elan 6000, Perkin
23 Elmer, USA) ¹⁶. Samples from *in-vitro* digestion were collected in tubes (10 ml) and 0.15 ml
24 of concentrated nitric acid (HNO₃ 65%) was added to preserve them. These samples were
25 analysed by the Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, Elan 6000, Perkin
26 Elmer, USA). Measurements were performed in duplicate.

27

28

1

2 *Phytate determination*

3 Approximately 10 mg of grain flour was extracted with 1 ml of 0.5N HCl containing 50 mg/l
4 cis-aconitate (internal standard) ¹⁷. The mixture was boiled in a water bath at 100°C for 15
5 min and then centrifuged at 14,000 g for 10 min. The supernatant was diluted 5x in millipore
6 water and analysed using HPLC (Dionex DX300, ICS2500 system, detector range of 10 µS)
7 using the column AS11 (ATC column + guard column). Detection was with suppressed
8 conductivity and the suppression was done with water at a flow rate of 5 ml/min. The eluent
9 and the elution times used are as follows: 0-5 min 5 mM NaOH ; 5-15 min 5-100 mM
10 NaOH ; 15-20 min 500 mM NaOH and 20-35 min 5 mM NaOH. A standard solution was
11 prepared in millipore water, that contains 5.0 mg/l NaNO₃ (Merck p.a.), 5.0 mg/l Na₂SO₄,
12 (Merck p.a.), 5.0 mg/l Oxalic acid.2H₂O (Merck p.a.), 10.0 mg/l Na₂HPO₄.2H₂O (Merck 6346
13 p.a.), 10 mg/l citric acid, H₂O (Merck K23524044 719 p.a.), 5.0 mg/l cis-aconitate (Aldrich
14 27194-2) and 10 mg/l IP₆.Na₁₂ (Sigma P3168 lot 102K0053). Measurements were performed
15 in triplicate.

16

17 *Total phenolics determination*

18

19 Total phenolic compounds (PC) were extracted from 50 mg of flour in 1.5 ml of
20 HCl/methanol (1% v/v) for 1 h under continuous stirring at room temperature. The mixture
21 was centrifuged at 5,000 g for 10 min and supernatant was removed. Next the pellet was re-
22 extracted as described above and supernatants were pooled ¹⁸. The PC were measured
23 following the method of Singleton and Rossi ¹⁹ modified as follows. 300 µl of extract were
24 added with 4.2 ml of distilled water, 0.75 ml of Folin-Ciocalteu's reagent (Merck, Germany)
25 and 0.75 ml of sodium carbonate solution (20% w/v). After incubation for 30 min the optical
26 density was measured at 760 nm using a spectrophotometer (*Shimadzu* UV 240, Kyoto,
27 Japan). Blanks were always freshly prepared, in which Folin-Ciocalteu's reagent was

1 replaced by water to correct for interfering compounds. Gallic acid (New Jersey, USA) was
2 used as standard and the results were expressed as gallic acid equivalent per g of samples.

3

4 *Crude protein, ash and colour measurement*

5

6 Crude protein (N x 6.25) and ash were determined according to the AOAC method ²⁰. The
7 colour of grain samples was measured with a Minolta CR-210 portable chromameter
8 (Illuminant D65 CIE 1976) standardized with a standard white tile (Y = 94.8, x = 0.315 and y
9 = 0.3324). The L, a*, b* values were recorded (L= whitness index, a*= redness index , b*=
10 yellowness index) and the browning index was calculated as: $BI = 100-L$ ²¹. Because adding
11 water to flour may lead to colour changes as observed in our own experiments (data not
12 shown), we took this into account in the interpretation of data on cooked flour.

13

14 *Statistical analysis*

15

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

20

21

1 **Results and discussion**

2 *Variation in household dibou processing*

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

20

21 *Impact on total Fe and Zn content*

22 The variations in Fe and Zn content of sorghum grain during *dibou* preparation following the
23 three process scenarios are presented in Table 2. The Zn content of the grain remains constant
24 throughout the process with a slight increase after cooking, possibly due to contamination
25 from the metallic cooking pot. The Fe and ash (in scenarios 2 and 3) content also increased

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

16

17 *Impact on phytate and total phenolics*

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

1 spoiled grains, and attrition dust. The wet cleaning appeared to be more efficient in removing
2 these exogenous particles.

3

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

13

14 *Impact on in-vitro solubility of Fe and Zn*

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

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

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

19

20

1 **Conclusion**

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

12

13

14

15

16

17

18 **Aknowledgement**

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

23

24

25

26

1 **References**

- 2 1. Svanberg U and Lorri W, Fermentation and nutrient availability. *Food Control* **8**:319-327
3 (1997).
- 4 2. Frossard E, Bucher M, Mächler F, Mozafar A and Hurrell R, Potential for increasing the
5 content and bioavailability of Fe, Zn, and Ca in plants for human nutrition. *Journal of the*
6 *Science of Food and Agriculture* **80**:861-879 (2000).
- 7 3. Graf E, Chemistry and application of phytic acid: An overview. In Graf E.(ed.) Phytic acid:
8 Chemistry and application. *Pilatus Press*: Minneapolis, MN, pp 1-21 (1986).
- 9 4. Duodu KG, Taylor JRN, Belton PS, and Hamaker BR, Factors affecting sorghum protein
10 digestibility. *Journal of Cereal Science* **38**:117-131 (2003).
- 11 5. Hounhouigan DJ, La valorisation des céréales locales pour les marchés urbains en Afriques
12 de l'Ouest: les atouts, contraintes et perspectives, in *Food based approaches for a healthy*
13 *nutrition in West Africa; Proceedings of the 2nd international workshop*, Brouwer I.D.,
14 Traore A.S. and Treche S. University of Ouagadougou, Ouagadougou; Institute for
15 Development Research, Montpellier; Wageningen University, Wageningen; Food and
16 Agriculture Organization, Rome, Ouagadougou, pp 359-368 (2004).
- 17 6. Kayodé APP, Adégbidi A, Linnemann AR, Nout MJR and Hounhouigan DJ, Quality of
18 farmer's varieties of sorghum and derived foods as perceived by consumers in Benin. *Ecology*
19 *of Food and Nutrition* **44**:271-294 (2005).
- 20 7. Brekke O L and Kwolek WF, Corn dry-milling: cold-tempering and degermination of corn of
21 various initial moisture content. *Cereal Chemistry* **46**:545-559 (1969).
- 22 8. Reichert RD, Sorghum dry milling. In sorghum in the eighties : *Proceedings of the*
23 *international symposium on sorghum, 2-7 November 1981, Patancheru, AP, India (Vol.2).*
24 *ICRISAT, Patancheru, AP, India. pp. 547-564* (1982).
- 25 9. Sahay KM, Evaluation of a general purpose abrasive mill for pearling of coarse cereals and
26 dehusking of pulses. *International Journal of Food Science and Technology* **25**:220-225
27 (1990).
- 28 10. Beta T, Rooney LW, Marovatsanga L and Taylor JRN, Phenolic compounds and kernel
29 characteristics of Zimbabwean sorghums *Journal of the Science of Food and Agriculture*
30 **79**:1003-1010 (1999).
- 31 11. Fretzdorff B and Weiper D, Phytic acid in cereals: phytase in rye and rye products. *Zeitschrift*
32 *fur Lebensmittel Untersuchung und –Forschung* **82**:287-293 (1986).
- 33 12. Wanasundera JPD and Ravindran G, Effect of cooking on the nutrient and antinutrient
34 contents of yam tubers (*Dioscorea alata* and *Dioscorea esculenta*). *Food Chemistry* **45**:247-
35 250 (1992).
- 36 13. Mahgoub SEO and Elhag SA, Effect of milling, soaking, malting, heat-treatment and
37 fermentation on phytate level of four Sudanese sorghum cultivars. *Food Chemistry* **61**:77-80
38 (1998).
- 39 14. Duhan A, Khetarpaul N and Bishnoi S, Content of phytic acid and HCl-extractability of
40 calcium, phosphorus and iron as affected by various domestic processing and cooking
41 methods. *Food Chemistry* **78**:9-14 (2002).
- 42 15. Kiers JL, Nout MJR and Rombouts FM, In vitro digestibility of processed and fermented soya
43 bean, cowpea and maize. *Journal of the Science of Food and Agriculture* **80**:1325-1331
44 (2000).
- 45 16. Temminghof, E. (2000). Soil and Plant Analysis. Part 3. Plant analysis procedures.
46 Wageningen University Environmental Sciences, Wageningen (1997).
- 47 17. Bentsink L, Yuan K, Koornneef M and Vreugdenhil D, The genetics of phytate and
48 phosphate accumulation in seeds and leaves of *Arabidopsis thaliana*, using natural variation.
49 *Theoretical and Applied Genetics* **106**: 1234-1243 (2003).
- 50 18. Cia T, Ejeta G and Butler L G, Screening for grain polyphenol variants from high-tanin
51 sorghum somaclones. *Theoretical and Applied Genetics* **90**:211-220 (1995).
- 52 19. Singleton VL and Rossi JA, Colorimetry of total phenolic compounds with phosphomolybdic
53 phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**:144-158
54 (1965).

- 1 20. American Association of Cereal Chemists, Approved Method of the AACC, *St Paul*
2 *Minnesota 8th edition* (1984).
- 3 21. Mestres C, Dorthe S, Akissoe N and Hounhouigan DJ, Prediction of sensorial properties
4 (colour and taste) of amala, a paste from yam flour of West Africa, through flour biochemical
5 properties. *Plant Foods for Human Nutrition* **59**:93-99 (2004).
- 6 22. Kayodé APP, Linnemann AR, Hounhouigan DJ, Nout MJR and van Boekel MAJS, Genetic
7 and environmental impact on iron, zinc and phytate in food sorghum grown in Benin
8 (submitted).
- 9 23. Jambunathan R, Improvement of the nutritional quality of sorghum and pearl millet. *Food*
10 *and Nutrition Bulletin* **2**:11-16 (1980).
- 11 24. Traoré T, Mouquet C, Icard-Vernière C, Traoré AS and Trèche S, Changes in nutrient
12 composition, phytate and cyanide contents and α -amylase activity during cereal malting in
13 small production units in Ouagadougou (Burkina Faso). *Food Chemistry* **88**:105-114 (2005).
- 14 25. Reddy NR, Occurrence, distribution, content, and dietary intake of phytate. In N.R. Reddy
15 and S. K. Sathe (eds.), *Food phytates* (pp. 25-52). Boca Raton: CRC Press (2002).
- 16 26. Barroga CF, Laurena AC and Mendoza EMT. Polyphenols in mung bean (*Vigna radiata* (L.)
17 Wilczek): determination and removal. *Journal of Agricultural and Food Chemistry* **33**:1006-
18 1009 (1985).
- 19 27. Ekpenyong TE, Effect of cooking on polyphenolics content of some Nigerian legumes and
20 cereals. *Nutrition Reports International* **31**:561-565 (1985).
- 21 28. Matuscheck E, Towo E and Svanberg U, Oxidation of polyphenols in phytate-reduced high-
22 tannin cereals: *Effect* on different phenolic groups and on in vitro accessible Iron. *Journal of*
23 *Agricultural and Food Chemistry* **49**:5630-5638 (2001).
- 24 29. Lestienne I, Icard-Vernière C, Mouquet C, Picq C and Trèche S, Effect of soaking whole
25 cereal and legume seeds on iron, zinc and phytate contents. *Food Chemistry* **89**:421-425
26 (2005).
- 27
- 28
- 29

1 Table 1 : Frequency of use of unit process operations involved in *dibou* preparation by 52
 2 households from 2 regional communities in Northern Benin (in % of n respondents)
 3
 4

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

5

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±0.2a	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 mass¹, Fe and Zn during *dibou* preparation

	Mass (kg dm) ²	Fe (g dm)	Zn (g dm)
Scenario 1 (<i>dry cleaning - grinding - cooking</i>)			
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 (<i>dry cleaning - grinding - sieving - cooking</i>)			
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 (<i>wet cleaning - grinding - sieving - cooking</i>)			
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

¹The 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.

²Means ± 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 = [$\{\text{IVS Fe (mg kg}^{-1} \text{ dm)}\} / \{\text{Total Fe (mg kg}^{-1} \text{ dm)}\}$] x 100; ³ In Vitro Soluble Ratio Zn = [$\{\text{IVS Zn (mg kg}^{-1} \text{ dm)}\} / \{\text{Total Zn (mg kg}^{-1} \text{ dm)}\}$] 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; ³BI : 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.

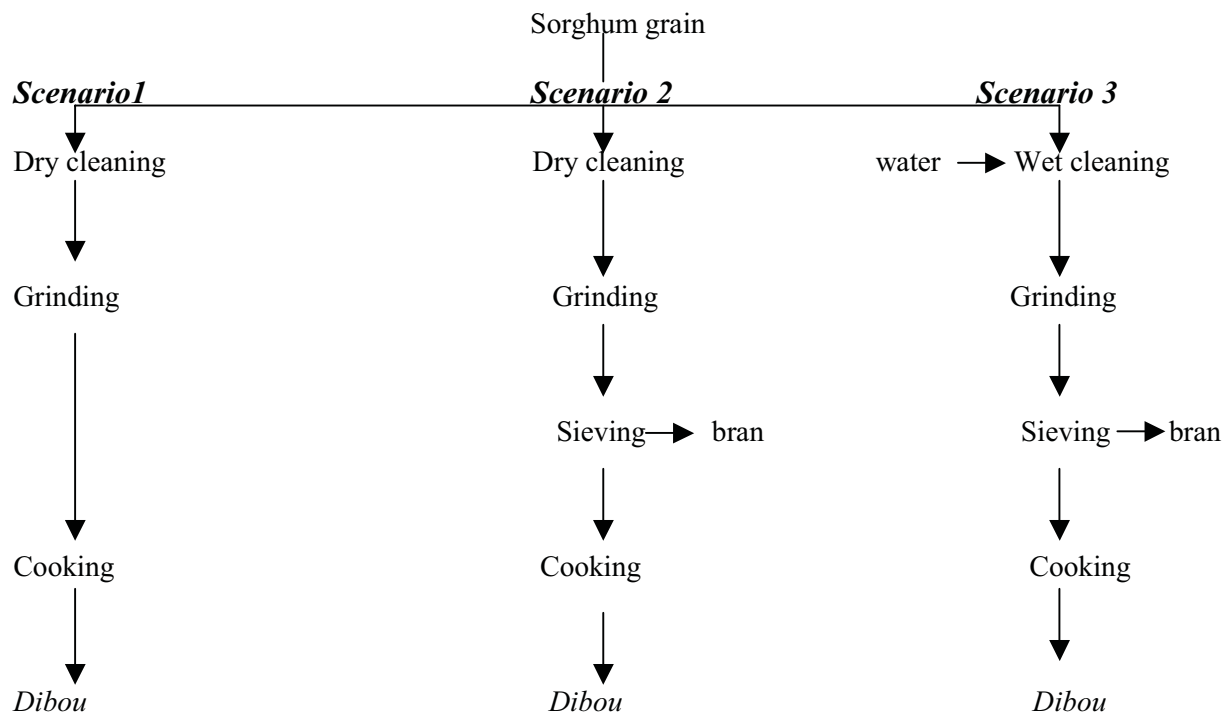


Figure 1: Process diagrams showing the 3 scenarios of *dibou* production