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Impact of traditional processing on proximate composition, folate, minerals, phytate, and alpha-galacto-oligosaccharide contents of two West African cowpea (*Vigna unguiculata L. Walp*) based doughnuts

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1 Abstract

2 Doughnuts made from cowpea, a highly nutritious pulse, are frequently consumed in West 3 Africa. As processing may affect their nutritional composition, cowpea processing into two 4 doughnut types (ata and ata-doco) was characterized, and samples collected from 12 producers 5 in Cotonou, Benin. Proximate composition, folate, minerals, phytate, and alpha-galacto-6 oligosaccharide contents were determined in the raw material, intermediate products, and 7 doughnuts. Mass balance was assessed during at a production to monitor folate and alpha-8 galacto-oligosaccharides distribution, and to determine what steps most influenced their 9 concentration. Ata was prepared with dehulled-soaked seeds, and ata-doco with whole or 10 partially dehulled, non-soaked and dry-milled seeds. After both types of doughnuts production, 11 lipid content increased by 11-33 times compared with raw seeds, due to oil absorption during 12 deep-frying. Milling led to an increase of iron content by 50-57% (ata) and 21-75% (ata-doco 13 production). Alpha-galacto-oligosaccharide contents decreased by 22-57% after whipping 14 during ata-doco, but not during ata production. The mass balance assessment showed 15 significant reductions of folate (-50%) and alpha-galacto-oligosaccharides (-33%) after dehulled 16 seed washing and soaking during at production. This study showed that the impact of 17 traditional processing on the nutritional value of cowpea-based doughnuts is strong, but highly 18 variable depending on the doughnut type and producers' practices.

Keywords: Black-eyed pea, Akara, Raffinose, Stachyose, Verbascose, Vitamin B9, Iron
 contamination, Oil absorption, Mass balance, Food analysis, Food composition, Benin.
 21

22 1. Introduction

23 Encouraging the production and consumption of pulses worldwide is important because of their 24 numerous beneficial characteristics in terms of environmental impact and nutritional value. 25 Indeed, pulses are an important source of plant proteins in low and middle income countries, 26 where access to animal proteins is often limited (FAO, 2016). They also have high contents of 27 fibre, and of some important minerals and vitamins. Cowpea (Vigna unguiculata L. Walp) also 28 called black-eyed pea, black-eyed bean, or field peas (Unal et al., 2006), is the most produced 29 and consumed pulse in West Africa. It is well adapted to high temperature and drought. Thus, 30 this crop represents a good option to face climate changes (Carvalho et al., 2017) and to 31 improve food security in low and middle income countries. In West African countries, cowpea is 32 consumed alone, or in combination with cereals (Akinyele and Akinlosotu, 1987). As pulses 33 have an amino acid pattern complementary to that of cereal grains, such pulse-cereal 34 association leads to a protein mix of good nutritional quality (Jayathilake et al., 2018). Cowpea 35 seeds are also a good source of health-promoting components (Awika and Duodu, 2017), such 36 as phenolic acids and flavonoids. Moreover, they contain soluble and insoluble fibre, and many 37 other functional compounds, including B group vitamins, like folate (vitamin B9) and thiamine 38 (vitamin B1) (Gonçalves et al., 2016). Cowpea seeds are also a good source of minerals 39 (potassium, phosphorus, calcium, sulphur, magnesium, iron, zinc, manganese, and copper) 40 (Harmankaya et al., 2016). However, they contain some chelating components (e.g. phytate or 41 polyphenols) that reduce the mineral bioavailability (Rogério et al., 2014). Another major 42 constraint is the presence of alpha-galacto-oligosaccharides in the seeds and in most cowpea-

based dishes. These indigestible oligosaccharides cause abdominal discomfort and flatulence
(Ndubuaku et al., 1989). Winham and Hutchins, (2011) reported that many people avoid
consuming some pulses due to the intestinal gas they generate.

46 Cowpea is widely consumed in Benin, a country located in West Africa, in various dishes 47 prepared following combinations of unit operations that can affect their nutritional value. 48 Madode et al., (2011) identified 18 different cowpea traditional dishes in Benin among which 10 49 (abla, abobo, adjagbé, adowè, ata, ataclè, atassi, ata-doco, magni-magni, djongoli) are popular 50 in the southern cities of the country. Most dishes are prepared from seeds, but cowpea leaves 51 are sometimes used for the preparation of sauces. Among the seed-based dishes, in Benin and 52 in most other West-African countries, the cowpea-based doughnuts known as ata (with a light-53 brown colour crust) are widely consumed for breakfast or as a snack at any time of the day, in 54 combination with cereal porridges, fried yam, sweet potato fries, akassa or lio (i.e. cooked 55 fermented maize dough) (Madode et al., 2011). Ata (Benin), akara (Nigeria), koose (Ghana) 56 (Madode et al., 2011) and even acarajé (Brazil) (Rogério et al., 2014) refer to the same product: 57 a doughnut prepared by deep-frying in oil from a batter made from dehulled and soaked 58 cowpea seeds. This dish is prepared daily at home and by street food vendors in urban and 59 rural areas (Henshaw et al., 2000). In Benin, ata-doco is another type of cowpea doughnut that 60 differs from ata by the dark-brown colour of the crust due to partial or no dehulling. The unit 61 operations during the preparation of these doughnuts can reduce the micronutrient contents 62 due to fractionation, diffusion, thermal degradation during frying, or oxidation reactions. This

reduction could compromise the nutritional interest of cowpea seeds, when eaten under theform of doughnuts.

65 Previous studies have described the production of ata and ata-doco doughnuts – or similar 66 products – and analysed their proximate composition, mineral content and phytate content 67 (Madode et al., 2011; Rogério et al., 2014; Feitosa et al., 2015). However, they reported only 68 the nutritional composition of the final products – as consumed – and did not focus on the 69 effect of the intermediary processing steps. Moreover, to our knowledge, no study has 70 monitored the changes in folate and alpha-galacto-oligosaccharide contents during ata and ata-71 doco production, although cowpea seeds are rich in this vitamin and this anti-nutritional factor 72 (Gonçalves et al., 2016).

73 The first objective of the present work was to characterize the traditional processing of cowpea 74 into ata and ata-doco, as carried out in small production units in Benin and its effects on the 75 proximate composition, micronutrient (minerals, vitamin B9) and anti-nutritional factor 76 contents (phytate and alpha-galacto-oligosaccharide) in the raw material, intermediate and 77 final products, depending on the used processing variants. The second objective was to identify 78 the processing steps that are responsible for the loss of folate and alpha-galacto-79 oligosaccharides during ata production. Therefore, a mass balance assessment was performed 80 in small production units to monitor the folate and alpha-galacto-oligosaccharides distribution 81 in the different fractions generated at each step.

82

83 2. Materials and methods

- 84 **2.1. Materials**
- 85

2.1.1. Collection and pre-treatment of cowpea seeds

86 The characterization of the traditional processing of cowpea (Vigna unquiculata L. Walp) into 87 ata and ata-doco was performed, using a commonly consumed local cowpea cultivar named 88 Atchawe-Tola (white seeds), grown in Azowlissè (a rural town in Ouémé department, Benin) in 89 2017, and provided by a farmer under the control of the International Institute of Tropical 90 Agriculture (IITA, Benin). The assessment of the folate and alpha-galacto-oligosaccharide losses 91 at each unit operation of cowpea processing for ata production (mass balance assessment) was 92 carried using the same cowpea cultivar grown in 2019 and purchased from a local farmer in the 93 same department. Before use, cowpea seeds were cleaned by removal of damaged grains, 94 insects and stones, placed at -20°C for one week to destroy insect eggs, and then stored in a dry 95 place at room temperature until processing.

96

2.1.2. Traditional processing into ata or ata-doco and sampling

97 All the producers used the same batch of raw material: cowpea seeds (1 kg) were provided to
98 12 cowpea-based doughnut's producers randomly selected in different areas of Cotonou
99 (Benin) for processing into ata (n=6) and ata-doco (n=6), according to their own traditional
100 procedures.

For laboratory analyses, 33 intermediates and 14 final products were sampled: *cleaned and dehulled seeds, soaked grits, whipped dough* and *ata doughnuts* (during ata production); *flour, whipped dough* and *ata-doco doughnuts* (during ata-doco production) (Fig. 1). Duration of

soaking, whipping and frying as well as frying temperatures were recorded during thepreparation of both doughnuts.

106 The collected samples were immediately placed in an icebox for transportation to the 107 laboratory where they were stored at -20°C.

108

109

• Freeze-drying and milling

2.1.3. Sample preparation for composition analysis

Fifty grams of each frozen sample were freeze-dried and milled using a laboratory mill IKA M20
(IKA Labortechnik, Staufen, Germany). All freeze-dried and milled samples were then stored at
4°C until analysis (proximate composition, minerals, alpha-galacto-oligosaccharide and phytate
contents).

114

Cryo-milling

Twenty grams of each frozen sample were milled under liquid nitrogen using a laboratory mill IKA M20 (IKA Labortechnik, Staufen, Germany) to obtain an homogeneous powder immediately placed into an opaque bag to avoid humidification through ambient air, and stored at -20°C.
Cryo-milled samples were used for folate analysis.

119 **2.1.4.** Reagents, enzymes, reference materials and standards

The test kit used for the analysis of total dietary fibre (K-TDFR kits), as well as verbascose (purity>95%), were purchased from Megazyme (Bray Co. Wicklow, Ireland). The following chemicals were purchased from Carlo Erba (Val de Reuil, France): petroleum ether, ethanol, hydrogen peroxide, and nitric acid. Hydrochloric acid (37%) was purchased from Fluka (Honeywell Fluka, Muskegon, USA). The following chemicals were purchased from Sigma-

Aldrich (St Louis, USA): α-amylase from Aspergillus oryzae (A9857), protease from Streptomyces
griseus (P5147), sodium ascorbate (A4034), galactose (G-0750), glucose (G-7528), fructose (F2543), sucrose (S-7903), raffinose pentahydrate (206679), and Sodium hydroxide. The
conjugase from chicken pancreas (P2002) was purchased from R-Biopharm (Berlin, Germany),
and Folic Acid Casei Medium was purchase form Difco (Sparks, MD, USA). Reference materials
were purchased from IRMM (Geel, Belgium): White cabbage (BCR-679) and Wholemeal flour
(BCR-121).

132

133 **2.2. Methods**

134

2.2.1. Composition analysis

135

• Proximate composition and energy determination

136 Total dry matter (DM), crude protein, lipid and ash contents were determined using standard 137 methods (AOAC, 1995). Soluble and insoluble dietary fibre contents were determined with the 138 Megazyme K-TDFR kit, as described by Njoumi et al., (2019). Before analysis, all frozen doughnuts 139 were lyophilized and cut into small pieces. Each sample was reduced into powder in petroleum 140 ether using an ultra-turrax IKA T10 (IKA Labortechnik, Staufen, Germany), then filtered, and 141 dried overnight at ambient temperature to obtain a defatted powder. Powdered samples of ata 142 and ata-doco doughnuts were pooled based on the processing variant used by the producers 143 (Fig.1), yielding 5 pooled samples: 2 for ata processing and 3 for ata doco processing. After that, 144 samples were successively digested with α -amylase, protease and amyloglucosidase, and then 145 filtered. After filtration, extracts were precipitated with 95% ethanol warmed at 70°C (extract/ethanol; 1/4; v/v) for soluble fibre determination, and the filtration residue was used for insoluble dietary fibre quantification. Available carbohydrates (AC) were calculated by difference: AC (DM) = (100 g – (protein content + lipid content + total dietary fibre + ash content)). Energy was determined using the Atwater coefficients: 4 kcal.g⁻¹ for proteins and available carbohydrates, 9 kcal.g⁻¹ for lipids, and 2 kcal.g⁻¹ for dietary fibres (FAO, 2003).

151

• Minerals

152 Total iron, zinc, calcium, and magnesium were determined in cowpea seeds, intermediate products and doughnuts, as described by Njoumi et al., (2018). Briefly, after wet mineralization of 153 the freeze-dried samples with a mixture of hydrogen peroxide/nitric acid (1/7; v/v), using an 154 155 Ethos 1 microwave digestion system (Milestone, Sorisole, Italy), minerals were determined by 156 optical emission spectrometry using an inductively coupled plasma - optical emission 157 spectrometer ICP-OES 5100 apparatus (Agilent Technologies, Les Ulis, France). White cabbage (BCR-679) was used as reference material, with certified mineral contents of 5.5 ± 0.3 mg iron .100 g 158 1 DM , 8.0 ± 0.3 mg zinc .100 g $^{-1}$ DM, 777 ± 65 mg calcium .100 g $^{-1}$ DM, and 136 ± 13 mg 159 magnesium .100 g⁻¹ DM. The average mineral contents obtained during this study were 5.7 ± 0.4 160 mg iron .100 g⁻¹ DM , 7.0 \pm 2.7 mg zinc .100 g⁻¹ DM, 798 \pm 49 mg calcium .100 g⁻¹ DM, and 128 \pm 161 24 mg magnesium .100 g^{-1} DM (on n=10 analyses). 162

163

• Folate (B9 vitamin) quantification

Total folate was determined by enzymatic extraction and microbiological assay, using the method described by Kariluoto and Piironen, (2009) and adapted by Bationo et al., (2020). Briefly, 0.5 g of each sample was digested with α -amylase (20 mg.mL⁻¹ in 1% sodium ascorbate) and

conjugase (100 mg.mL⁻¹ in ultrapure water) at 37°C for 3h. After this first enzymatic treatment, 167 protease (3 mg.mL⁻¹ in 1% sodium ascorbate) was added and the mixture was incubated at 37°C 168 169 for 1h. Sample extracts were diluted and pipetted into a 96-well microtiter plate (Greiner Bio-170 One, Frickenhausen, Germany). Folic Acid Casei Medium inoculated with Lactobacillus rhamnosus ATCC 7469 was added to all wells and incubated at 37°C for 18h. Wholemeal flour 171 172 (BCR-121) was used as reference material to check the accuracy of the total folate 173 quantification method using the same analytical procedure. After incubation, folate was quantified using a microplate reader (Tecan, Infinite M200 PRO, Lyon, France) set at 590 nm to 174 175 measure the turbidity due to bacterial growth.

The certified total folate content for the reference material BCR-121 (whole wheat flour) was 50 \pm 7 µg.100 g⁻¹ DM. The average total folate content obtained during this study was 47 \pm 6 µg.100 g⁻¹ DM (average value for n=49 analyses).

179

• Phytic acid

Phytic acid (IP6) content was determined by high-performance anion-exchange
chromatography (Dionex, Sunnyvale, CA, USA) after sample extraction in 0.5M HCl (0.1/5; w/v)
at 100 °C for 6 min, according to the method described by Lestienne et al., (2005).

183

• Alpha-galacto-oligosaccharides

Alpha-galacto-oligosaccharides (raffinose, stachyose and verbascose) and their potential degradation products (saccharose, galactose, fructose, glucose) were extracted as described by Njoumi et al., (2019) with some modifications. For samples used to assess the effect of processing variants, 3 mL of ethanol (78%; v/v) was added to 80 mg of each lyophilized sample

for extraction in two rounds at 80 °C for 20 min. Supernatants were dehydrated in a vacuum centrifuge concentrator RC 10 (Thermo Fisher Scientific, Asheville, NC, USA), and the dry extracts were dissolved in 2 mL of pure water, before injection.

The samples collected for the mass balance assessment were not lyophilized, and 80-700 mg were used, depending on the dry matter content of each sample (higher quantity was required for samples with low dry matter content). Alpha-galacto-oligosaccharides extraction followed the same procedure, but samples of soaking water were injected directly after filtration through cellulose acetate filters (0.2 μm).

196 Alpha-galacto-oligosaccharides separation and quantification were carried out by high 197 performance anion exchange chromatography with pulsed amperometric detection using a Thermo Scientific[™] Dionex[™] ICS-6000 HPIC[™] System (Thermo Fisher Scientific, Sunnyvale, CA, 198 199 USA) equipped with a Dionex[™] CarboPac[™] PA210-Fast-4 µm analytical column (150 × 4 mm) 200 and a Dionex[™] CarboPac[™] PA210-Fast–4 µm guard column (30 × 4 mm). Elution was performed at 30 °C with isocratic flux at 0.8 mL.min⁻¹ of 12 mmol.L⁻¹ KOH. Injection volume ranged from 10 201 202 to 25 µL. Sugars were identified by comparison of the retention time with that of appropriate 203 standards (galactose, glucose, fructose, sucrose, raffinose pentahydrate, verbascose).

204

• Micronutrient quantification in defatted doughnuts

To assess the "passive dilution effect" due to oil uptake during frying, the micronutrient content
of "defatted samples" was calculated on a dry matter basis with the following equation:

$$C_{df} = \frac{C_0}{100 - F_S} * 100$$

207

208 Where C_{df} is the micronutrient content in the defatted sample, C_0 the micronutrient 209 concentration in the sample diluted with oil, and F_s the fat content (% DM) of the sample. This 210 formula allows assessing the part of decrease caused by other phenomena than oil uptake, such 211 as enzymatic degradation, diffusion, oxidation or thermal inactivation.

212

2.2.2. Mass balance assessment

213 To identify the unit operations that have the greatest effect on folate and alpha-galacto-214 oligosaccharide contents, a mass balance assessment was carried during ata production in small 215 processing units. Ata doughnuts were produced by three producers using the dry dehulling 216 processing in Cotonou (Benin). Batches of 2 kg of cowpea seeds were provided to each 217 producer. The weight of all containers used for collecting the fractions obtained at each step 218 was recorded, as well as the weight of the produced fractions (raw material, intermediate 219 products, by-products, and doughnuts). The samples collected for the different fractions were 220 also weighed. These weights were taken into account in the calculation to determine the exact 221 mass at each processing step. The DM contents of all samples were measured, and combined 222 with the folate and sugar contents to calculate the amounts of folate (in µg) and alpha-galacto-223 oligosaccharide (in mg) in the raw material, intermediate products, and final products. Then, 224 the folate and alpha-galacto-oligosaccharide distribution in the produced fractions was 225 expressed as the percentage of their initial quantity in the raw material.

226 **2.3. Statistical analysis**

Data were analysed with the Statgraphics Centurion 16.2 software (FranceStat, Neuilly, France).
To describe the nutritional composition of intermediate and final products, means and standard

deviations were calculated, and differences between samples were assessed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test, with a significance level set at p<0.05.

To assess the effect of the different processing factors on the nutrient contents of ata and atadoco doughnuts, two-level (ata) and three-level (ata-doco) nested ANOVAs were carried out. For example, the linear model for a two-way unbalanced nested arrangement, with two nested factors (A: fixed factor with i levels, and B: random factor with j levels) was:

$$y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{k(ij)}$$

236

where: Y_{ijk} is the kth observation within the jth level of factor B within the ith level of factor A; μ is the overall mean; α_i is the fixed effect due to the ith level of factor A; $\beta_{j(i)}$ is the random effect due to the jth level of factor B nested within the ith level of A; and $\varepsilon_{k(ij)}$ is the residual error of the observation Y_{ijk} .

For ata doughnuts, "dehulling type" was used as fixed factor (two levels: dry dehulling and wet dehulling) and nested within the factor "producer" (six levels: 1 to 6). For ata-doco, the first factor was "milling type" (two levels: direct milling and crushing before milling) nested within the factor "use of NaHCO₃" (two levels: with and without NaHCO₃) that in turn, was nested within the factor "producer" (six levels: 1-6). The variance associated with each factor was calculated and expressed as a percentage.

General linear models were used to evaluate the association between processing parameters and doughnut nutrient content (proximate composition, mineral, folate, phytate and alphagalacto-oligosaccharide):

$$y = \beta_0 + \beta_1 * (factor 1) + \beta_2 * (factor 2) + ... + \beta_k * (factor k)$$

250 where β_0 is the constant, and β_{1-k} the coefficients associated with each factor. 251 The factors considered were the processing parameters: number of washing of the grits after 252 dehulling, soaking duration, soaking water volume/seed volume ratio, number of milling cycles, 253 whipping duration, and frying time and temperatures (ata); type of milling, whipping duration, 254 quantity of NaHCO₃ added to the dough, and frying time and temperatures (ata-doco). For both 255 doughnut types, the effect of frying parameters on the mineral contents was not assessed. 256 Models were adjusted, by removing the not significant factors (based on a statistical 257 significance level set at p<0.05).

258

259 **3.** Results and discussion

260 **3.1. Characterization of ata and ata-doco processing**

261 During the traditional ata processing (all variants), the main steps were dehulling, soaking, wet 262 milling, whipping, and frying (Fig. 1). Ata doughnuts were prepared from dehulled seeds 263 obtained by dry or wet dehulling. Wet dehulling was performed manually using seeds 264 previously wetted with tap water for softening the seed coat. For dry dehulling, cowpea seeds 265 were coarsely milled into grits using local mills equipped with two metallic grindstones. The 266 crushing step was followed by washing and manual rubbing to remove the seed coats. Grits 267 obtained after dehulling was soaked and milled into a dough using the same type of mills 268 previously used for crushing. During ata production, the mean duration of cowpea grits soaking, 269 dough whipping and frying were 22 ± 11 min, 23 ± 15 min, and 5 ± 2 min respectively, for 1 kg

270 of cowpea processed. The mean frying temperature varied between 135°C at the beginning 271 (mean T_0) and 173°C at the end of cooking (mean T_F). For ata-doco production, the main steps 272 were dry milling, whipping and frying. Whole seeds were directly milled (direct milling variant) 273 or crushed grossly and then winnowed before milling into flour (crushing before milling variant) 274 (Fig. 1). Then, water was added to the flour to obtain a dough. Among the five producers of ata-275 doco doughnuts who used the direct milling variant, two added NaHCO₃ to the dough as 276 leavening agent during whipping. Whatever the variant, the mean whipping and frying times 277 were 23 \pm 9 min and 5 \pm 2 min respectively, with frying temperatures ranging between 162 278 (mean T₀) and 193 °C (mean T_F). The variability observed for the frying temperature for both 279 doughnuts could be due to the different energy sources used by the producers (firewood, 280 charcoal and wood shavings).

281

3.2. Effect of traditional processing variants on ata and ata-doco nutritional composition

283

3.2.1. Proximate composition of ata and ata-doco doughnuts

The proximate composition of cowpea seeds, ata and ata-doco is presented in Table 1. The mean protein content of the raw material was 22 g.100 g⁻¹ DM, which is in the range reported by Madode et al., (2012) (*i.e.* 22 to 30 g.100 g-1 DM) for thirty cowpea varieties grown in West Africa. Protein content in cowpea seeds can vary depending on the cultivar (Jayathilake et al., 2018), environmental conditions and agronomic practices (Dakora and Belane, 2019; Sebetha et al., 2014). The lipid content of the cowpea seeds in our study was 1.6 g.100 g⁻¹ DM, which is rather low, as usually observed in pulses (Dilis and Trichopoulou, 2009). The total dietary fibre content of cowpea seeds was high (15 g.100 g⁻¹ DM) and on average 83% of fibres were insoluble. Other studies reported similar results for dietary fibre content in cowpea seeds (Eashwarage et al., 2017; Sreerama et al., 2012).

294 The protein, ash, and total (soluble and insoluble) dietary fibre contents were significantly 295 lower in ata than ata-doco doughnuts (p < 0.05). As expected, lipid content increased sharply 296 during the doughnut preparation. The mean lipid content of ata doughnuts was significantly higher than that of ata-doco (48 and 28 g.100 g^{-1} DM for ata and ata-doco, respectively). 297 298 McWatters (1983) obtained similar results for doughnuts prepared from dehulled-soaked cowpea and whole cowpea flour (that presented lipid contents of 32 g.100 g^{-1} DM and 21 g.100 299 g^{-1} DM, respectively). The higher oil absorption by doughnuts prepared with dehulled and 300 301 soaked seeds compared with those prepared with whole flour could be due to the dough 302 particle size that is influenced by the milling conditions. Indeed, wet milling generates finer 303 particles than dry milling, and products with a predominantly fine particle size distribution have 304 a very high oil absorption capacity (Moreira et al., 1997; Singh et al., 2004). Another possible cause of the difference of oil absorption could be the higher soluble fibre content of the dough 305 306 used for ata-doco preparation. By swelling in the presence of water, soluble fibre can increase 307 the dough viscosity and this could influence oil absorption. Indeed, Lee and Inglett, (2007) 308 showed that the incorporation of oat β -glucans into a wheat flour batter reduces oil absorption 309 during deep-frying, because of the high water retention capacity and viscosity generated by 310 these polysaccharides. Moreover, a study on poori, a traditional wheat-based doughnut 311 consumed in India, showed that the use of fibre-rich flour (4.2% total dietary fibres, 1.3%

soluble dietary fibres) allowed reducing the oil content by 20% compared with the control sample Yadav and Rajan, (2012). In this study, the major consequences of oil absorption during frying were the passive reduction of the proportions of other nutrients in both doughnut types and the strong increase of the energy value (Table 1). Thus, the energy value of ata doughnuts was significantly higher than that of ata-doco (p <0.05), due to the high lipid content of ata doughnuts, which was twice higher than the one of ata-doco doughnuts.

318 The producers' practices influenced the proximate composition of the doughnuts. The two-level 319 nested ANOVA showed that 85-97% of the variability observed in ata doughnut proximate 320 composition (dry matter, protein, lipid and ash contents) was due to inter-producer variability 321 (Table 1S, supplementary data). A specific analysis of the processing parameters that can lead 322 to this producer-related variability highlighted a negative effect of dry dehulling, soaking 323 duration, and initial frying temperature (T_0) on protein content (p<0.05, Table 2S, 324 supplementary data). Ash content was negatively correlated with soaking duration and 325 positively correlated with the number of milling cycles (p<0.05, Table 2S, supplementary data).

For ata-doco doughnuts, 55% and 60% of the observed variability of protein and lipid contents was explained by inter-producer variability, respectively (Table 3S, supplementary data). Protein content variability was related also to the "milling type" factor (27%), with higher mean protein content in doughnuts prepared using the "direct milling" than the "crushing before milling" variant (17 g.100 g⁻¹ DM vs 16 g.100 g⁻¹ DM, p<0.05). Moreover, by studying the effect of processing parameters on protein content, a negative correlation was observed with the variant "crushing before milling" and the initial frying temperature (p<0.05, Table 2S,

supplementary data). The analysis of the impact of the processing parameters showed that frying duration and initial frying temperature (T_0) were positively correlated with the lipid content. The nested ANOVA showed that the ash content of ata-doco doughnuts was mainly influenced by the factors "use of NaHCO₃" and "producer" (that contributed to 44% and 37% of the variance, respectively).

3.2.2. Changes in micronutrients and anti-nutritional factors during ata and ata-doco

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Minerals • **Minerals**

production

Cowpea seeds contained appreciable amounts of minerals with nutritional interest, particularly 342 343 iron and magnesium (Table 2). Iron content of doughnuts increased during processing for both 344 types. This increase in iron content of both doughnut types could be due to contamination from 345 various possible sources throughout processing. Some authors reported that exogenous iron 346 from pots and mills can be added to the food during processing (Adish et al., 1999; Hama-Ba et 347 al., 2019). Greffeuille et al., (2011) observed this contamination after maize milling into flour. 348 The processing steps before frying did not lead to a significant reduction of zinc, calcium and 349 magnesium content in both doughnut types. However, a passive decrease in mineral contents 350 was observed after frying due to oil absorption by the dough. Indeed, the calculation of the 351 mineral content in defatted ata doughnuts showed zinc, calcium, and magnesium contents (5.2 mg.100 g⁻¹, 83 mg.100 g⁻¹ and 188 mg.100 g⁻¹ DM, respectively), similar to that in the whipped 352 353 dough. The mean iron, zinc, calcium and magnesium contents calculated in defatted ata-doco

were 4.1 mg.100 g⁻¹, 90 mg.100 g⁻¹ and 168 mg.100 g⁻¹ DM, respectively. These results show the oil dilution effect on mineral contents.

356 For ata doughnuts, the nested ANOVA (Table 1S, supplementary data) showed that more than 357 60% of the variability of iron, calcium, and magnesium content was due to the inter-producer 358 variability. Conversely, the variability of zinc content was very low and not related to any factor. 359 The analysis of the different processing parameters that could cause this producer-related 360 variability of iron, calcium, and magnesium contents in ata doughnuts showed a negative effect 361 of the number of dehulled-seed washing steps on iron content (p<0.05, Table 2S, 362 supplementary data), and a negative effect of the number of dehulled-seed washing steps and 363 of soaking duration on magnesium and calcium content (p<0.05, Table 2S, supplementary data). 364 These decrease in iron, calcium and magnesium contents of ata doughnuts could be due to 365 leaching from the grits during washing.

For ata-doco, the iron content variability was related to the factor "milling type" (84% of 366 367 variance, Table 3S, supplementary data), with higher iron content in the "crushing before milling" than in the "direct milling" variant (28 \pm 10 mg.100 g⁻¹ DM vs. 5.5 \pm 1 mg.100 g⁻¹), which 368 369 shows the influence of processing types on iron contamination. Zinc content variability was also 370 low and more related to the factor "producer" (50%). This producer-related variability was not 371 caused by the processing parameters. The calcium and magnesium content variability was 372 mainly due to the factor "use of NaHCO3" (52% of the variance for calcium, and 56% for 373 magnesium). Indeed, the calcium and magnesium contents of ata-doco doughnuts prepared with NaHCO₃ were lower than those of doughnuts prepared without NaHCO₃ (58 \pm 1 mg.100 g⁻¹ 374

375 DM vs. 68 ± 6 mg.100 g⁻¹ DM for calcium and 113 ± 3 mg.100 g⁻¹ DM vs. 124 ± 7 mg.100 g⁻¹ DM
376 for magnesium).

377

• Folate

The total folate content of the initial raw material was $362 \pm 33 \mu g.100 g^{-1}$ DM (Table 2). 379 Hoppner and Lampi, (1993) found a similar folate content of 367 \pm 28 µg.100 g⁻¹ DM in cowpea 380 381 seeds purchased from a local market in Canada. During the production of both doughnut types, 382 a reduction in folate content was observed after each step. The mean total folate content of ata-doco doughnuts was significantly higher than that of ata doughnuts (177 \pm 36 μ g.100 g⁻¹ DM 383 vs. 111 \pm 29 µg.100 g⁻¹ DM). This difference in the mean folate content of ata and ata-doco 384 385 doughnuts could be due to the absence of soaking during ata-doco production. Indeed, a 386 negative effect of soaking duration on folate content was observed during ata production 387 (Table 2S, supplementary data for details). Different studies showed the effect of the soaking 388 on folate content in legumes. Arcot et al. (2002) observed a reduction of 31% of total folate 389 after soaking of soybeans overnight at room temperature. In their study, the recovery of folate 390 from soaked soybeans and the soaking water was almost equal to 100% of the initial amount in 391 the soybeans showing that the loss was due to leaching into the soaking water. During soaking 392 at 30°C of whole cowpea seeds, Coffigniez et al. (2019) also observed folate diffusion, but at a 393 much lower rate than at 60°C and 95°C. In our study, the negative effect of soaking duration 394 observed could be due to an increased diffusion due to the high surface area generated by the 395 small size of the grits.

The nested ANOVA showed that the total folate content variability for ata was mainly related to the factor "producer" (91% of variance, Table 1S, supplementary data). Indeed, the total folate content was negatively correlated with the dry dehulling variant, the number of dehulled seed washing steps, and the soaking duration (p<0.05, Table 2S, supplementary data). For ata-doco, 87% of the observed variability was due to inter-producer variability (Table 3S, supplementary data), possibly due to the NaHCO₃ amount added to the dough (positive correlation, p < 0.05, Table 2S supplementary data).

403

404 • Phytate

405 Phytate content of both doughnut types was only slightly affected by processing (Table 2), 406 although it decreased at some production steps. During ata production, dehulling did not have 407 any effect on phytate contents. This could be due to phytic acid localization in the seed. In 408 pulses, phytic acid is stored in globoids, located in protein bodies that are in the seed 409 cotyledons (Reddy, 2002). Thus, the dehulling is not an efficient way to reduce phytate in 410 cowpea seeds. Similarly, soaking did not significantly reduce the phytate content of dehulled 411 seeds. Lestienne et al., (2005) found that soaking at 30°C for 24h significantly reduced phytate 412 content in millet, maize, rice and soybean, but not in cowpea. Ologhobo and Fetuga, (1984) 413 observed a reduction of 20-28% in phytate contents in three cowpea cultivars after soaking at 414 27°C for 3 days. Thus, the lack of effect observed in our study may be due to the short soaking 415 duration (between 9 and 38 min). In our study, phytate content in the dough used for ata 416 preparation decreased by 11% and 6% during the whipping step compared with soaked seeds,

417 for the wet and dry dehulling variants, respectively. This small effect of whipping on phytate 418 content was also observed during ata-doco preparation. The use of NaHCO₃ during ata-doco 419 processing did not affect phytate contents. The mean final phytate content was significantly higher in ata-doco than in ata doughnuts (656 mg.100 g⁻¹ DM for ata-doco vs. 526 mg.100 g⁻¹ 420 421 DM for ata). The variability in the doughnut phytate content was mostly related to the factor 422 "producer" (63% and 75% of the variance for ata and ata-doco, respectively). For ata, this 423 producer-related variability might be due to the number of grits washing steps and the duration 424 of soaking that were negatively correlated with phytate content (p < 0.05, Table 2S, 425 supplementary data). For ata-doco, the inter-producer variability was not explained by the 426 processing parameters.

427

428

Alpha-galacto-oligosaccharides

The total alpha-galacto-oligosaccharide content in cowpea seeds reached 3554 mg.100 g^{-1} DM, 429 430 and stachyose content was 8 to 10 times higher than that of verbascose and raffinose, as 431 already reported for other legume seeds (Njoumi et al., 2019). The changes in alpha-galacto-432 oligosaccharide content during ata and ata-doco processing are shown in Figure 2. During ata 433 production, raffinose (27%) and verbascose (28%) content increased after wet dehulling. 434 Sreerama et al., (2010) studied alpha-galacto-oligosaccharides distribution in cotyledon, 435 embryonic axis and seed coat of two pulse species: chickpea (*Cicer arietinum L.*) and horse gram 436 (Macrotyloma uniflorum L. Verdc.). They found higher concentrations of these oligosaccharides 437 in the cotyledon, but stachyose was present in higher amounts also in the embryonic axis. This

438 could explain the increase observed after wet dehulling because the removal of the seed coat, 439 which does not contain raffinose and verbascose, could lead to a passive increase of these 440 compounds in dehulled seeds. After dehulling during ata preparation, a decrease of 15-20% in 441 the content of the three alpha-galacto-oligosaccharide forms was observed up to the "whipped 442 dough" step, whatever the variant. The reduction after whipping was more significant (total 443 alpha-galacto-oligosaccharide content was halved) during ata-doco processing. This reduction 444 most probably results from the alpha-galactosidase activity in cowpea seeds (Coffigniez et al., 445 2018) or provided by exogenous factors, such as Aspergillus niger (Somiari and Balogh, 1992). Bulgarelli et al., (1988) reported that A. niger is among the predominant microbiological 446 447 populations in the dough used for akara (i.e. ata) preparation. After frying, the difference in alpha-galacto-oligosaccharide content of both doughnuts was compensated by the higher oil 448 449 absorption in ata, and the mean total alpha-galacto-oligosaccharide contents of ata and atadoco doughnuts were 2695 \pm 792 mg.100 g⁻¹ DM and 2680 \pm 255 mg.100 g⁻¹ DM, respectively. 450 451 When calculated in defatted doughnuts, the total alpha-galacto-oligosaccharide content was significantly higher in defatted at than in ata-doco (4623 mg.100 g⁻¹ DM vs. 3713 mg.100 g⁻¹ 452 453 DM, respectively).

Variability in the total alpha-galacto-oligosaccharide content was mostly caused by the factor "producer" (>97%) in ata doughnuts and by the factors "producer" (37%) and "use of NaHCO₃" (33%) in ata-doco. Specifically, the total mean alpha-galacto-oligosaccharide content was higher in ata-doco doughnuts prepared without than with NaHCO₃ (2764 mg.100 g⁻¹ DM vs. 2427 mg.100 g⁻¹ DM).

459

3.3. Mass balance of dry matter, folate and alpha-galacto-oligosaccharide during ata production

462 **3.3.1.** Dry matter

463 Dry matter losses and gains were observed during at a processing based on the dry dehulling 464 process (variant presented on the left in Fig. 1) (Fig. 3). The dehulling step (i.e. coarse milling 465 followed by washing and manual rubbing) resulted in an average loss of 16% of the dry matter 466 (8.6% due to diffusion into the washing water and 7.1% from the seed coats). Only one among 467 the three producers performed two cycles of crushed-seed washing, but this did not result in a 468 significant difference compared with the other producers because the second soaking water 469 contained only 2% of the initial dry matter. After soaking, the amount of dry matter in the grits 470 was about 78% of the initial dry matter of cowpea seeds. After milling, on average, 60% of the 471 initial dry matter was recovered as dough, and 12% was found in the water used for mill rinsing. 472 The addition of the mill rinsing water to the dough, to adjust the dough consistency during 473 whipping, allowed recovering, on average, 3% of dry matter. Oil absorption by the whipped 474 dough during frying was highly variable, resulting in a mean dry matter gain of about $27 \pm 16\%$.

475

476 **3.3.2.** Folates

The initial total folate content of the cowpea seeds used for the mass balance assessment was 274 μ g.100 g⁻¹ DM (Table 3), which was lower than that of the cowpea seeds used for the study of the processing variants (Table 2). Analysis of the distribution of the initial amount of folate in

480 the different fractions generated during at a production (Fig. 3) showed that approximately 24% 481 of the folate present in the cowpea seeds was found in the washing water and seed coat during 482 the washing step. Indeed, it has been shown that folate vitamers in cowpea seeds are mainly 483 concentrated in the embryonic axis (Coffigniez et al., 2019). As after dehulling and washing, the 484 embryonic axis is discarded, this could explain part of the folate loss. Moreover, dehulling leads 485 to the loss of fine particles from cotyledon, which are also washed out. After the soaking step, a 486 loss of 26% of the initial folate was recorded and only 4% was in the soaking water. The other 487 22% might be due to folate degradation. Indeed, some of the folate vitamers are very sensitive 488 to pH and oxygen (Strandler et al., 2015; Xue et al., 2011). Low pH and presence of oxygen can 489 lead to oxidative degradation or cause conversion to other forms, sometimes without vitaminic 490 activity (Coffigniez et al., 2019). In this study, milling resulted in an additional average loss of 491 13% of the initial folate. The addition of the mill rinsing water, which contained about 6% of the 492 initial folate, to the dough during whipping did not result in a significant difference between 493 freshly milled dough (41 % of the initial folate) and whipped dough (42%). Frying also strongly 494 affected folate contents. However, the main part of the decrease observed was related to oil 495 absorption by the dough during frying, and only approximately 10% of the decrease could be 496 explained by thermal degradation.

- 497
- 498

3.3.3. Alpha-galacto-oligosaccharides

The initial amounts of raffinose, stachyose and verbascose were reduced by 5%, 12% and 10%,
respectively, after dehulling (Fig. 3). Moreover, 22%, 32% and 36% of the initial amounts of

501 raffinose, stachyose and verbascose, respectively, were found in the seed coats and washing 502 water. After grits soaking, the amount of initial alpha-galacto-oligosaccharides found in the 503 soaking water and soaked grits (the two fractions generated after this step) allowed deducing 504 that 18 to 33% of the initial alpha-galacto-oligosaccharides were lost due to degradation. 505 Moreover, an important increase of the initial amounts of galactose, glucose and fructose, 506 which are alpha-galacto-oligosaccharide degradation products, was simultaneously observed 507 (Fig. 1S, supplementary data). The initial amount of these sugars increased by 23-fold for 508 galactose, 33-fold for glucose, and 14-fold for fructose. The higher decrease in alpha-galacto-509 oligosaccharide content of the whipped doughs used for ata-doco (half the one of ata) 510 observed during the study of the traditional processing variants could be explained by this 511 higher production of galactose observed after grits soaking during ata production. Indeed, 512 alpha-galactosidase activity can be reduced by the presence in the medium of some inhibitors, 513 particularly galactose (Coffigniez et al., 2018). According to Dey and Pridham, (1969), 6.2 $mmol.L^{-1}$ and 25 $mmol.L^{-1}$ of galactose inhibit alpha-galactosidase activity by 70% and 90% 514 515 respectively. Indeed, in our study, the galactose content of the raw material was 0.3 mmol.L⁻¹, and increased to 8.4 \pm 1 mmol.L⁻¹ in the soaked grits, which were milled into dough (Fig.1S, 516 517 supplementary data). After soaking, residual losses of alpha-galacto-saccharide were observed 518 up to the frying step.

519

520

3.4. Potential contribution of ata and ata-doco to the recommended nutrient intakes

521 In West Africa, cowpea-based doughnuts are consumed for breakfast and as a snack food. The 522 average adult serving is 3-4 doughnuts, which corresponds to approximately 100 g. The 523 consumption of 100 g of ata and ata-doco provides 8.2 ± 0.9 g and 9.3 ± 0.8 g of protein (Table 524 4S, supplementary data). For an adult weighing 65 kg, such serving of ata and ata-doco would 525 contribute to 15 and 17% respectively of the recommended nutrient intake (RNI) for protein, 526 which is estimated at 54 g of protein/day (FAO and OMS, 2007). The dietary fibre intake 527 associated to the consumption of 100 g of both doughnuts would be around 4.9 ± 0.9 g and 5.9 \pm 0.6 g for ata and ata-doco respectively. These values represent 16 to 20% of the daily fibre 528 529 recommended intake which is estimated at 30g per day by (ANSES, 2016). This serving could 530 also provide 8% and 13% of the iron RNI, 11% and 11% of the zinc RNI, and 20% and 26% of the 531 magnesium RNI for ata and ata-doco, respectively. Folate intake from 100 g of ata and ata-doco 532 would represent 16% and 24% of the folate RNI that is estimated at 400 µg/day for adults (FAO-533 WHO, 2004). Based on these results both doughnuts can be regarded as a source of protein, 534 dietary fibre, magnesium and folate (Codex, 1997). Calcium intake from both doughnut types is 535 low (less than 4% of the RNI) (Table 5S, supplementary data). However, this serving also 536 provides high amounts of lipids, much more elevated for ata $(28 \pm 5 \text{ g})$ than for ata-doco $(15 \pm 3 \text{ g})$ 537 g).

538

539 **4.** Conclusion

540 This study highlighted the complex effects of traditional processing on the proximate 541 composition and mineral, folate, phytate, and alpha-galacto-oligosaccharide contents of two

542 cowpea-based doughnuts, and their variability in function of the doughnut type and processing 543 variant. Part of these effects could be attributed to some processing parameters (e.g. soaking 544 duration, milling type), but the role of the producers' technological know-how remained 545 significant, hindering the overall understanding of the whole processing effect. Mineral content 546 was only slightly affected by the processing. Although folate losses were significant, the folate 547 content in the final products was enough to qualify these doughnuts as a source of folate. 548 Globally, ata-doco showed a better nutritional value than ata, with higher protein, fibre and 549 folate and lower lipid contents. This work allowed identifying the processing steps that 550 influence the nutritional quality of cowpea-based doughnuts, with the perspective of optimizing 551 their preparation in the future. A study in controlled conditions could help to better understand 552 and master the factors leading to folate and alpha-galacto-oligosaccharide losses due to 553 degradation (around 20%) during soaking. The important oil absorption observed during frying 554 led to a passive reduction of the other nutrient contents. This high lipid intake counterbalances 555 the nutritional interest of cowpea-based doughnuts. Therefore, more studies are also needed 556 to determine the processing conditions to limit oil absorption.

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721 Figure Captions

Figure 1: Diagram showing the different steps of cowpea seed processing into ata and ata-doco.
 ¹: Mean initial temperature (T0); ²: Mean final temperature (TF); ³: [Min value T0 - Max value TF]; *: indicates sampling points; n: number of producers; circle with grey outline: inputs; grey circle: by-products; circle with black outline: raw material and intermediate products; rectangular frame: different processing steps (light orange for ata and light green for ata-doco).

Figure 2: Raffinose, stachyose and verbascose content (on a dry matter basis) during ata and ata-doco) processing. n: number of producers, Different superscript letters correspond to significant difference between samples (p<0.05, Newman-Keuls test was used for post-hoc comparison).

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Figure 3: Dry matter, folate and alpha-galactoside distribution in the different production fractions during the traditional processing of ata (calculated using the dry weight of each fraction and component content; data are the mean values for the samples collected from three producers). Oil: proportion of dry matter gained due to oil absorption during frying.



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Table 1: Proximate composition of the cowpea seeds and doughnuts (ata and ata-doco) according to different processes

	Process	Dry matter	Protein	Fat	Available carbohydrate**	Ash	Dietary fibres			Energy
Products		(g/100g)	(g/100g DM)					(Kcal/100g)		
							IDF	SDF	TDF	
Cowpea seeds		89 ± 0.3	22.0 ± 1.0	1.6 ±0.0	58 ± 1.1	3.3 ±0.0	12.4 ± 1.0	2.5 ± 0.4	14.9 ± 1.2	309 ±1
Ata	Wet dehulling (n=3)	58 ± 1.0 ^a	14.0 ±1.4 ^a	42.7 ± 2.0 ^b	31 ±3.4 ^{ab}	2.6 ±0.9 ^a	8.4 ± 0.4 ^b	1.2 ±0.3 ^a	9.6 ±0.4 ^b	337 ±12 ^b
	Mechanical dry dehulling (n=3)	61 ± 9.7 ^a	14.2 ± 3.2 ^a	52.9 ± 13 ^c	24 ±16 ^a	2.9 ±0.2 ^a	5.2 ± 0.6 ^a	1.3 ±0.4 ^a	6.5 ±0.3 ^a	388 ± 36 [°]
Ata-doco	Direct milling (+) NaHCO ₃ (n=2)	54 ± 3.6 ^a	17.1 ± 1.2 ^b	32.4 ±2.4 ^a	36 ±3.1 ^b	3.4 ±0.3 ^a	8.6 ± 1.1 ^b	2.2 ±0.3 ^b	10.8 ± 1.5 ^b	285 ±17 ^a
	Direct milling (-) NaHCO₃ (n=3)	53 ± 3.5 ^a	17.9 ±1.0 ^b	25.6 ±5.5 ^a	40 ± 6.6 ^b	5.4 ±1.4 ^b	9.0 ± 0.9 ^b	2.3 ±0.4 ^b	11.3 ±0.8 ^b	254 ±8 ^a
	Crushing before milling (n=1) *	58 ± 4.4 ^a	15.8 ±0.8 ^{ab}	27.1 ±2.5 ^a	41 ± 2.2 ^b	6.0 ±1.0 ^b	8.3 ± 0.6 ^b	1.7 ±0.6 ^{ab}	10.0 ± 1.2 ^b	283 ±30 ^a
Ata vs. Ata- doco	P-value	ns	0.0005	<0.00001	0.002	<0.00001	0.02	0.0013	0.0028	<0.00001
	Ata	59 ± 6.8	14.1 ± 2.4	47.8 ±10	27 ± 12	2.8 ±0.6	7.1 ± 1.8	1.2 ± 0.3	8.3 ± 1.7	362 ±37
	Ata-doco	56 ± 4.3	16.9 ±1.3	27.8 ± 4.6	39 ± 4.7	5.1 ±1.5	8.6 ± 0.8	2.1 ± 0.5	10.7 ± 1.2	273 ±18

n: number of producers

IDF: Insoluble dietary fibres; SDF: soluble dietary fibres; TDF: Total dietary fibres

Results are the mean ± standard deviations (samples were collected from the indicated number of producers and analysed in duplicate). *: Samples were collected from this producer on three different days. **: Values were obtained by calculation.

Values in a same column with different superscript letters are significantly different (p < 0.05, Newman-Keuls test was used for post-hoc comparison).

Dish	Processing	Products	Iron (mg/100g DM)	Zinc (mg/100g DM)	Calcium (mg/100g DM)	Magnesium (mg/100g DM)	Phytate (mg/100g DM)	Folate (µg/100g DM)
Ata	Wet dehulling (n=3)	Raw cowpea seeds Dehulled seeds Soaked grits Whipped dough Ata	$\begin{array}{c} 5.8 \pm 0.0 \\ 4.5 \pm 0.1 \\ 4.5 \pm 0.2 \\ 11.5 \pm 6.8 \\ 8.9 \pm 3.0 \end{array} \begin{array}{c} a \\ bc \\ ab \\ \end{array}$	$\begin{array}{c} 3.9 \pm 0.0 \\ 3.9 \pm 0.1 \\ 4.0 \pm 0.1 \\ 3.6 \pm 0.3 \\ 2.3 \pm 0.4 \end{array} \begin{array}{c} c \\ c \\ a \\ a \end{array}$	$\begin{array}{c} & & d \\ 78 \pm 1 & c \\ 61 \pm 12 & c \\ 65 \pm 9 & c \\ 86 \pm 23 & d \\ 44 \pm 9 & ab \end{array}$	$\begin{array}{c} & & c \\ 163 \pm 1 & b \\ 144 \pm 2 & b \\ 143 \pm 2 & b \\ 135 \pm 8 & b \\ 91 \pm 17 & a \end{array}$	$\begin{array}{c} & & & & cd \\ 979 & \pm 81 & d \\ 1049 & \pm 82 & d \\ 1044 & \pm 72 & d \\ 871 & \pm 99 & b \\ 562 & \pm 101 & a \end{array}$	$\begin{array}{c} 362 \pm 33 \\ 318 \pm 43 \\ 261 \pm 23 \\ 195 \pm 21 \\ 98 \pm 29 \end{array}^{e}$
	Mechanical dehulling (n=3)	Dehulled seeds Soaked grits Whipped dough Ata	$\begin{array}{c} & \text{a} \\ 4.6 \pm 0.2 \\ 4.7 \pm 0.4 \\ 13.4 \pm 6.7 \\ 7.1 \pm 2.5 \end{array}$	$\begin{array}{c} 3.5 \pm 0.3 \\ 3.9 \pm 0.1 \\ 4.0 \pm 0.1 \\ 2.7 \pm 0.5 \end{array}^{c}$	$\begin{array}{c} & bc \\ 53 \pm 3 & bc \\ 55 \pm 5 & c \\ 60 \pm 2 & a \\ 37 \pm 11 & \end{array}$	$ \begin{array}{c} b \\ 140 \pm 5 \\ 139 \pm 6 \\ 137 \pm 7 \\ 92 \pm 22 \\ \end{array} $	$\begin{array}{c} & & & cd \\ 988 & \pm 69 & cd \\ 982 & \pm 38 & cd \\ 919 & \pm 63 & bc \\ 519 & \pm 105 & a \end{array}$	$\begin{array}{cccc} 305 \pm 22 & d \\ 293 \pm 38 & d \\ 204 \pm 30 & b \\ 113 \pm 27 & a \end{array}$
Ata-doco	Direct milling with NaHCO3 (n=2)	Raw cowpea seeds Flour Whipped dough Ata-doco	$\begin{array}{c} & A \\ 5.8 \pm 0.0 \\ 7.5 \pm 1.4 \\ 8.4 \pm 2.3 \\ 5.5 \pm 0.6 \end{array}$	$\begin{array}{c} 3.9 \pm 0.0 \\ 3.9 \pm 0.2 \\ 4.2 \pm 0.0 \\ 3.0 \pm 0.0 \end{array} \begin{array}{c} \text{BC} \\ \text{C} \\ \text{A} \end{array}$	$\begin{array}{c} & DE \\ 78 \pm 1 & DE \\ 77 \pm 3 & F \\ 86 \pm 0 & A \\ 58 \pm 1 & \end{array}$	163 ± 1 F 153 ± 5 DE 153 ± 7 A 113 ± 2 A	$\begin{array}{c} 979 & \pm 81 \\ 940 & \pm 76 \\ 814 & \pm 14 \\ 601 & \pm 79 \end{array} \begin{array}{c} E \\ C \\ A \end{array}$	$\begin{array}{c} 362 \pm 33 \\ 273 \pm 41 \\ 180 \pm 44 \\ 207 \pm 46 \end{array} \begin{array}{c} F \\ D \\ AB \\ BC \\ \end{array}$
	Direct milling without NaHCO3 (n=3)	Flour Whipped dough Ata-doco	$\begin{array}{c} & A \\ 7.2 \pm 0.3 \\ 7.6 \pm 1.0 \\ 5.5 \pm 0.9 \end{array} \begin{array}{c} A \\ A \end{array}$	$\begin{array}{c} & & & & & & & & & \\ 4.1 \pm 0.1 & & & & & \\ 3.9 \pm 0.2 & & & & & \\ 3.0 \pm 0.2 & & & & & \end{array}$	DE 78 ± 5 86 ± 5 69 ± 7	160 ± 4 EF 156 ± 5 DE 127 ± 8 C	$\begin{array}{c} 929 & \pm 85 \\ 800 & \pm 42 \\ 641 & \pm 79 \end{array} \stackrel{E}{}$	302 ± 41 DE 306 ± 38 E 176 ± 36 AB
	Crushing + Milling (n=1)*	Flour Whipped dough At- doco	$\begin{array}{c} & & & & \\ 37.9 \pm 10 & & \\ 37.9 \pm 13 & & \\ 28.3 \pm 12 & & \\ \end{array}$	3.9 ± 0.0 BC 3.8 ± 0.2 B 2.9 ± 0.2 A	CD 75±8 84±6 66±6	DE 153 ± 3 149 ± 5 121 ± 5	$\begin{array}{c} 980 & \pm 47 \\ 876 & \pm 41 \\ 686 & \pm 45 \end{array}^{B}$	287 ± 36 232 ± 26 157 ± 10

Table 2: Changes in mineral, phytate and folate contents (on a dry matter basis) during ata and ata-doco processing

n: number of producers

In each sub-column, values with different superscript letters are significantly different (p < 0.05, Newman-Keuls test was used for post-hoc comparison). Results are the mean \pm standard deviation (samples were collected from the indicated number of producers). *: Samples were collected from this producer on three different days.

Fractions	Dry matter (g/100 g)	Folate (µg/100 g DM)	Raffinose (mg/100 g DM)	Stachyose (mg/100 g DM)	Verbascose (mg/100 g DM)
Cowpea seeds	89.5 ± 1.5 ^h	274 ± 9 ^c	469 ± 7 ^d	4150 ± 137 ^e	658 ± 19 ^c
Mix of grits and seed coats	90.7 ± 1.1 ^h	293 ± 15 ^{cd}	456 ± 37 ^d	3761 ± 195 ^{de}	614 ± 42 ^c
Washing water 1	2.3 ± 0.7 ^a	378 ± 28 ^{ef}	727 ± 132 ^e	7814 ± 749 ^f	1304 ± 190 ^d
Washing water 2*	0.7 ^a	348 ^{de}	797 ^f	11391 ^g	1931 ^e
Seed coats	20.2 ± 2.6 ^c	366 ± 49 ^{ef}	49 ± 11 ^a	330 ± 153 ^a	99 ± 46 ^a
Soaking water	1.0 ± 0.2^{a}	429 ± 147 ^f	963 ± 124 ^g	14840 ± 2527 ^h	2794 ± 559 ^f
soaked grits	43.0 ± 1.8 ^f	189 ± 15 ^b	342 ± 40 ^c	2825 ± 157 ^c	521 ± 45 ^c
Mill rinsing water	14.5 ± 3.1 ^b	152 ± 52 ^{ab}	336 ± 30 ^c	3290 ± 174 ^{cd}	608 ± 35 ^c
Freshly milled dough	38.8 ± 1.3 ^e	187 ± 21 ^b	340 ± 27 ^c	2952 ± 20 ^c	548 ± 22 ^c
Whipped dough	32.5 ± 1.8 ^d	183 ± 17 ^b	338 ± 41 ^c	3012 ± 174 ^c	562 ± 23 ^c
Ata doughnuts	54.4 ± 2.8 ^g	98 ± 8 ^a	214 ± 23 ^b	1965 ± 176 ^b	327 ± 42 ^b

Table 3 : Dry matter, total folate and alpha-galactoside content in the different fractions during at a processing

Values in a same column with different superscript letters are significantly different (p < 0.05, Newman-Keuls test was used for post-hoc comparison). Results are the mean ± standard deviation (samples were collected from 3 producers and analysed in triplicate for folate and in duplicate for alpha-galactoside and dry matter quantification).

*: Only producer P3 used two rounds of washing.