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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 ata 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 ata 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.

19 **Keywords: Black-eyed pea, Akara, Raffinose, Stachyose, Verbascose, Vitamin B9, Iron**  
20 **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-

43 based dishes. These indigestible oligosaccharides cause abdominal discomfort and flatulence  
44 (Ndubuaku et al., 1989). Winham and Hutchins, (2011) reported that many people avoid  
45 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

63 reduction could compromise the nutritional interest of cowpea seeds, when eaten under the  
64 form 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 unguiculata 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.

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

104 soaking, whipping and frying as well as frying temperatures were recorded during the  
105 preparation 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 **2.1.3. Sample preparation for composition analysis**

#### 109 **● Freeze-drying and milling**

110 Fifty grams of each frozen sample were freeze-dried and milled using a laboratory mill IKA M20  
111 (IKA Labortechnik, Staufen, Germany). All freeze-dried and milled samples were then stored at  
112 4°C until analysis (proximate composition, minerals, alpha-galacto-oligosaccharide and phytate  
113 contents).

#### 114 **● Cryo-milling**

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

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

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



125 Aldrich (St Louis, USA):  $\alpha$ -amylase from *Aspergillus oryzae* (A9857), protease from *Streptomyces*  
126 *griseus* (P5147), sodium ascorbate (A4034), galactose (G-0750), glucose (G-7528), fructose (F-  
127 2543), sucrose (S-7903), raffinose pentahydrate (206679), and Sodium hydroxide. The  
128 conjugase from chicken pancreas (P2002) was purchased from R-Biopharm (Berlin, Germany),  
129 and Folic Acid Casei Medium was purchase form Difco (Sparks, MD, USA). Reference materials  
130 were purchased from IRMM (Geel, Belgium): White cabbage (BCR-679) and Wholemeal flour  
131 (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  $\alpha$ -amylase, protease and amyloglucosidase, and then  
145 filtered. After filtration, extracts were precipitated with 95% ethanol warmed at 70°C

146 (extract/ethanol; 1/4; v/v) for soluble fibre determination, and the filtration residue was used  
147 for insoluble dietary fibre quantification. Available carbohydrates (AC) were calculated by  
148 difference:  $AC (DM) = (100 \text{ g} - (\text{protein content} + \text{lipid content} + \text{total dietary fibre} + \text{ash content}))$ .  
149 Energy was determined using the Atwater coefficients:  $4 \text{ kcal.g}^{-1}$  for proteins and available  
150 carbohydrates,  $9 \text{ kcal.g}^{-1}$  for lipids, and  $2 \text{ kcal.g}^{-1}$  for dietary fibres (FAO, 2003).

151 **● Minerals**

152 Total iron, zinc, calcium, and magnesium were determined in cowpea seeds, intermediate  
153 products and doughnuts, as described by Njoumi et al., (2018). Briefly, after wet mineralization of  
154 the freeze-dried samples with a mixture of hydrogen peroxide/nitric acid (1/7; v/v), using an  
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  
158 (BCR-679) was used as reference material, with certified mineral contents of  $5.5 \pm 0.3 \text{ mg iron .100 g}^{-1}$   
159  $^1 \text{ DM}$  ,  $8.0 \pm 0.3 \text{ mg zinc .100 g}^{-1} \text{ DM}$ ,  $777 \pm 65 \text{ mg calcium .100 g}^{-1} \text{ DM}$ , and  $136 \pm 13 \text{ mg}$   
160  $\text{magnesium .100 g}^{-1} \text{ DM}$ . The average mineral contents obtained during this study were  $5.7 \pm 0.4$   
161  $\text{mg iron .100 g}^{-1} \text{ DM}$  ,  $7.0 \pm 2.7 \text{ mg zinc .100 g}^{-1} \text{ DM}$ ,  $798 \pm 49 \text{ mg calcium .100 g}^{-1} \text{ DM}$ , and  $128 \pm$   
162  $24 \text{ mg magnesium .100 g}^{-1} \text{ DM}$  (on n=10 analyses).

163 **● Folate (B9 vitamin) quantification**

164 Total folate was determined by enzymatic extraction and microbiological assay, using the  
165 method described by Kariluoto and Piironen, (2009) and adapted by Bationo et al., (2020). Briefly,  
166 0.5 g of each sample was digested with  $\alpha$ -amylase ( $20 \text{ mg.mL}^{-1}$  in 1% sodium ascorbate) and

167 conjugase (100 mg.mL<sup>-1</sup> in ultrapure water) at 37°C for 3h. After this first enzymatic treatment,  
168 protease (3 mg.mL<sup>-1</sup> in 1% sodium ascorbate) was added and the mixture was incubated at 37°C  
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*  
171 *rhamnosus* ATCC 7469 was added to all wells and incubated at 37°C for 18h. Wholemeal flour  
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  
174 quantified using a microplate reader (Tecan, Infinite M200 PRO, Lyon, France) set at 590 nm to  
175 measure the turbidity due to bacterial growth.

176 The certified total folate content for the reference material BCR-121 (whole wheat flour) was  
177 50 ± 7 µg.100 g<sup>-1</sup> DM. The average total folate content obtained during this study was 47 ± 6  
178 µg.100 g<sup>-1</sup> DM (average value for n=49 analyses).

179 **● Phytic acid**

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

183 **● Alpha-galacto-oligosaccharides**

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

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

191 The samples collected for the mass balance assessment were not lyophilized, and 80-700 mg  
192 were used, depending on the dry matter content of each sample (higher quantity was required  
193 for samples with low dry matter content). Alpha-galacto-oligosaccharides extraction followed  
194 the same procedure, but samples of soaking water were injected directly after filtration  
195 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  
198 Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System (Thermo Fisher Scientific, Sunnyvale, CA,  
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  
201 at 30 °C with isocratic flux at 0.8 mL.min<sup>-1</sup> of 12 mmol.L<sup>-1</sup> KOH. Injection volume ranged from 10  
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**

205 To assess the “passive dilution effect” due to oil uptake during frying, the micronutrient content  
206 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  $\mu\text{g}$ ) 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**

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

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

232 To assess the effect of the different processing factors on the nutrient contents of ata and ata-  
233 doco doughnuts, two-level (ata) and three-level (ata-doco) nested ANOVAs were carried out.  
234 For example, the linear model for a two-way unbalanced nested arrangement, with two nested  
235 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  
237 where:  $Y_{ijk}$  is the  $k^{\text{th}}$  observation within the  $j^{\text{th}}$  level of factor B within the  $i^{\text{th}}$  level of factor A;  $\mu$  is  
238 the overall mean;  $\alpha_i$  is the fixed effect due to the  $i^{\text{th}}$  level of factor A;  $\beta_{j(i)}$  is the random effect  
239 due to the  $j^{\text{th}}$  level of factor B nested within the  $i^{\text{th}}$  level of A; and  $\varepsilon_{k(ij)}$  is the residual error of the  
240 observation  $Y_{ijk}$ .

241 For ata doughnuts, “dehulling type” was used as fixed factor (two levels: dry dehulling and wet  
242 dehulling) and nested within the factor “producer” (six levels: 1 to 6). For ata-doco, the first  
243 factor was “milling type” (two levels: direct milling and crushing before milling) nested within  
244 the factor “use of  $\text{NaHCO}_3$ ” (two levels: with and without  $\text{NaHCO}_3$ ) that in turn, was nested  
245 within the factor “producer” (six levels: 1-6). The variance associated with each factor was  
246 calculated and expressed as a percentage.

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

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

250 where  $\beta_0$  is the constant, and  $\beta_{1-k}$  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  $\text{NaHCO}_3$  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  $22 \pm 11$  min,  $23 \pm 15$  min, and  $5 \pm 2$  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  $\text{NaHCO}_3$  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  $T_0$ ) 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

## 282 **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**

284 The proximate composition of cowpea seeds, ata and ata-doco is presented in Table 1. The  
285 mean protein content of the raw material was  $22 \text{ g} \cdot 100 \text{ g}^{-1}$  DM, which is in the range reported  
286 by Madode et al., (2012) (*i.e.* 22 to 30  $\text{g} \cdot 100 \text{ g}^{-1}$  DM) for thirty cowpea varieties grown in West  
287 Africa. Protein content in cowpea seeds can vary depending on the cultivar (Jayathilake et al.,  
288 2018), environmental conditions and agronomic practices (Dakora and Belane, 2019; Sebetha et  
289 al., 2014). The lipid content of the cowpea seeds in our study was  $1.6 \text{ g} \cdot 100 \text{ g}^{-1}$  DM, which is  
290 rather low, as usually observed in pulses (Dilis and Trichopoulou, 2009). The total dietary fibre



291 content of cowpea seeds was high (15 g.100 g<sup>-1</sup> DM) and on average 83% of fibres were  
292 insoluble. Other studies reported similar results for dietary fibre content in cowpea seeds  
293 (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  
297 higher than that of ata-doco (48 and 28 g.100 g<sup>-1</sup> DM for ata and ata-doco, respectively).  
298 McWatters (1983) obtained similar results for doughnuts prepared from dehulled-soaked  
299 cowpea and whole cowpea flour (that presented lipid contents of 32 g.100 g<sup>-1</sup> DM and 21 g.100  
300 g<sup>-1</sup> DM, respectively). The higher oil absorption by doughnuts prepared with dehulled and  
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  
305 cause of the difference of oil absorption could be the higher soluble fibre content of the dough  
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  $\beta$ -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%

312 soluble dietary fibres) allowed reducing the oil content by 20% compared with the control  
313 sample Yadav and Rajan, (2012). In this study, the major consequences of oil absorption during  
314 frying were the passive reduction of the proportions of other nutrients in both doughnut types  
315 and the strong increase of the energy value (Table 1). Thus, the energy value of ata doughnuts  
316 was significantly higher than that of ata-doco ( $p < 0.05$ ), due to the high lipid content of ata  
317 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).

326 For ata-doco doughnuts, 55% and 60% of the observed variability of protein and lipid contents  
327 was explained by inter-producer variability, respectively (Table 3S, supplementary data).

328 Protein content variability was related also to the "milling type" factor (27%), with higher mean  
329 protein content in doughnuts prepared using the "direct milling" than the "crushing before  
330 milling" variant ( $17 \text{ g} \cdot 100 \text{ g}^{-1} \text{ DM}$  vs  $16 \text{ g} \cdot 100 \text{ g}^{-1} \text{ DM}$ ,  $p < 0.05$ ). Moreover, by studying the effect  
331 of processing parameters on protein content, a negative correlation was observed with the  
332 variant "crushing before milling" and the initial frying temperature ( $p < 0.05$ , Table 2S,

333 supplementary data). The analysis of the impact of the processing parameters showed that  
334 frying duration and initial frying temperature ( $T_0$ ) were positively correlated with the lipid  
335 content. The nested ANOVA showed that the ash content of ata-doco doughnuts was mainly  
336 influenced by the factors “use of  $\text{NaHCO}_3$ ” and “producer” (that contributed to 44% and 37% of  
337 the variance, respectively).

338

### 339 **3.2.2. Changes in micronutrients and anti-nutritional factors during ata and ata-doco** 340 **production**

#### 341 **• Minerals**

342 Cowpea seeds contained appreciable amounts of minerals with nutritional interest, particularly  
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  
352  $\text{mg}\cdot 100\text{ g}^{-1}$ , 83  $\text{mg}\cdot 100\text{ g}^{-1}$  and 188  $\text{mg}\cdot 100\text{ g}^{-1}$  DM, respectively), similar to that in the whipped  
353 dough. The mean iron, zinc, calcium and magnesium contents calculated in defatted ata-doco

354 were 4.1 mg.100 g<sup>-1</sup>, 90 mg.100 g<sup>-1</sup> and 168 mg.100 g<sup>-1</sup> DM, respectively. These results show the  
355 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.

366 For ata-doco, the iron content variability was related to the factor "milling type" (84% of  
367 variance, Table 3S, supplementary data), with higher iron content in the "crushing before  
368 milling" than in the "direct milling" variant (28 ± 10 mg.100 g<sup>-1</sup> DM vs. 5.5 ± 1 mg.100 g<sup>-1</sup>), which  
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 NaHCO<sub>3</sub>" (52% of the variance for calcium, and 56% for  
373 magnesium). Indeed, the calcium and magnesium contents of ata-doco doughnuts prepared  
374 with NaHCO<sub>3</sub> were lower than those of doughnuts prepared without NaHCO<sub>3</sub> (58 ± 1 mg.100 g<sup>-1</sup>

375 DM vs.  $68 \pm 6 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$  for calcium and  $113 \pm 3 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$  vs.  $124 \pm 7 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$   
376 for magnesium).

377

378 ● **Folate**

379 The total folate content of the initial raw material was  $362 \pm 33 \text{ } \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ DM}$  (Table 2).  
380 Hoppner and Lampi, (1993) found a similar folate content of  $367 \pm 28 \text{ } \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ DM}$  in cowpea  
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  
383 ata-doco doughnuts was significantly higher than that of ata doughnuts ( $177 \pm 36 \text{ } \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ DM}$   
384 vs.  $111 \pm 29 \text{ } \mu\text{g} \cdot 100 \text{ g}^{-1} \text{ DM}$ ). This difference in the mean folate content of ata and ata-doco  
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.

396 The nested ANOVA showed that the total folate content variability for ata was mainly related to  
397 the factor "producer" (91% of variance, Table 1S, supplementary data). Indeed, the total folate  
398 content was negatively correlated with the dry dehulling variant, the number of dehulled seed  
399 washing steps, and the soaking duration ( $p < 0.05$ , Table 2S, supplementary data). For ata-doco,  
400 87% of the observed variability was due to inter-producer variability (Table 3S, supplementary  
401 data), possibly due to the  $\text{NaHCO}_3$  amount added to the dough (positive correlation,  $p < 0.05$ ,  
402 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  $30^\circ\text{C}$  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  $27^\circ\text{C}$  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 NaHCO<sub>3</sub> during ata-doco  
419 processing did not affect phytate contents. The mean final phytate content was significantly  
420 higher in ata-doco than in ata doughnuts (656 mg.100 g<sup>-1</sup> DM for ata-doco vs. 526 mg.100 g<sup>-1</sup>  
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**

429 The total alpha-galacto-oligosaccharide content in cowpea seeds reached 3554 mg.100 g<sup>-1</sup> DM,  
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* (Somari and Balogh, 1992).  
446 Bulgarelli et al., (1988) reported that *A. niger* is among the predominant microbiological  
447 populations in the dough used for akara (i.e. ata) preparation. After frying, the difference in  
448 alpha-galacto-oligosaccharide content of both doughnuts was compensated by the higher oil  
449 absorption in ata, and the mean total alpha-galacto-oligosaccharide contents of ata and ata-  
450 doco doughnuts were  $2695 \pm 792 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$  and  $2680 \pm 255 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$ , respectively.  
451 When calculated in defatted doughnuts, the total alpha-galacto-oligosaccharide content was  
452 significantly higher in defatted ata than in ata-doco ( $4623 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$  vs.  $3713 \text{ mg} \cdot 100 \text{ g}^{-1}$   
453 DM, respectively).

454 Variability in the total alpha-galacto-oligosaccharide content was mostly caused by the factor  
455 "producer" (>97%) in ata doughnuts and by the factors "producer" (37%) and "use of  $\text{NaHCO}_3$ "  
456 (33%) in ata-doco. Specifically, the total mean alpha-galacto-oligosaccharide content was higher  
457 in ata-doco doughnuts prepared without than with  $\text{NaHCO}_3$  ( $2764 \text{ mg} \cdot 100 \text{ g}^{-1} \text{ DM}$  vs.  $2427$   
458  $\text{mg} \cdot 100 \text{ g}^{-1} \text{ DM}$ ).



459

460       **3.3. Mass balance of dry matter, folate and alpha-galacto-oligosaccharide during ata**  
461           **production**

462           **3.3.1. Dry matter**

463 Dry matter losses and gains were observed during ata 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**

477 The initial total folate content of the cowpea seeds used for the mass balance assessment was  
478  $274 \mu\text{g}\cdot 100 \text{ g}^{-1} \text{ DM}$  (Table 3), which was lower than that of the cowpea seeds used for the study  
479 of the processing variants (Table 2). Analysis of the distribution of the initial amount of folate in

480 the different fractions generated during its 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**

499 The initial amounts of raffinose, stachyose and verbascose were reduced by 5%, 12% and 10%,  
500 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  
514 mmol.L<sup>-1</sup> and 25 mmol.L<sup>-1</sup> of galactose inhibit alpha-galactosidase activity by 70% and 90%  
515 respectively. Indeed, in our study, the galactose content of the raw material was 0.3 mmol.L<sup>-1</sup>,  
516 and increased to 8.4 ± 1 mmol.L<sup>-1</sup> in the soaked grits, which were milled into dough (Fig.1S,  
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  $8.2 \pm 0.9$  g and  $9.3 \pm 0.8$  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  $4.9 \pm 0.9$  g and  $5.9$   
528  $\pm 0.6$  g for ata and ata-doco respectively. These values represent 16 to 20% of the daily fibre  
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  $\mu\text{g}/\text{day}$  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 ( $28 \pm 5$  g) than for ata-doco ( $15 \pm 3$   
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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563

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721 **Figure Captions**

722 **Figure 1:** Diagram showing the different steps of cowpea seed processing into ata and ata-doco.

723 <sup>1</sup>: Mean initial temperature (T0); <sup>2</sup>: Mean final temperature (TF); <sup>3</sup>: [Min value T0 - Max value

724 TF]; \*: indicates sampling points; n: number of producers; circle with grey outline: inputs; grey

725 circle: by-products; circle with black outline: raw material and intermediate products;

726 rectangular frame: different processing steps (light orange for ata and light green for ata-doco).

727

728 **Figure 2:** Raffinose, stachyose and verbascose content (on a dry matter basis) during ata and

729 ata-doco) processing. n: number of producers, Different superscript letters correspond to

730 significant difference between samples ( $p < 0.05$ , Newman-Keuls test was used for post-hoc

731 comparison).

732

733 **Figure 3:** Dry matter, folate and alpha-galactoside distribution in the different production

734 fractions during the traditional processing of ata (calculated using the dry weight of each

735 fraction and component content; data are the mean values for the samples collected from

736 three producers). Oil: proportion of dry matter gained due to oil absorption during frying.

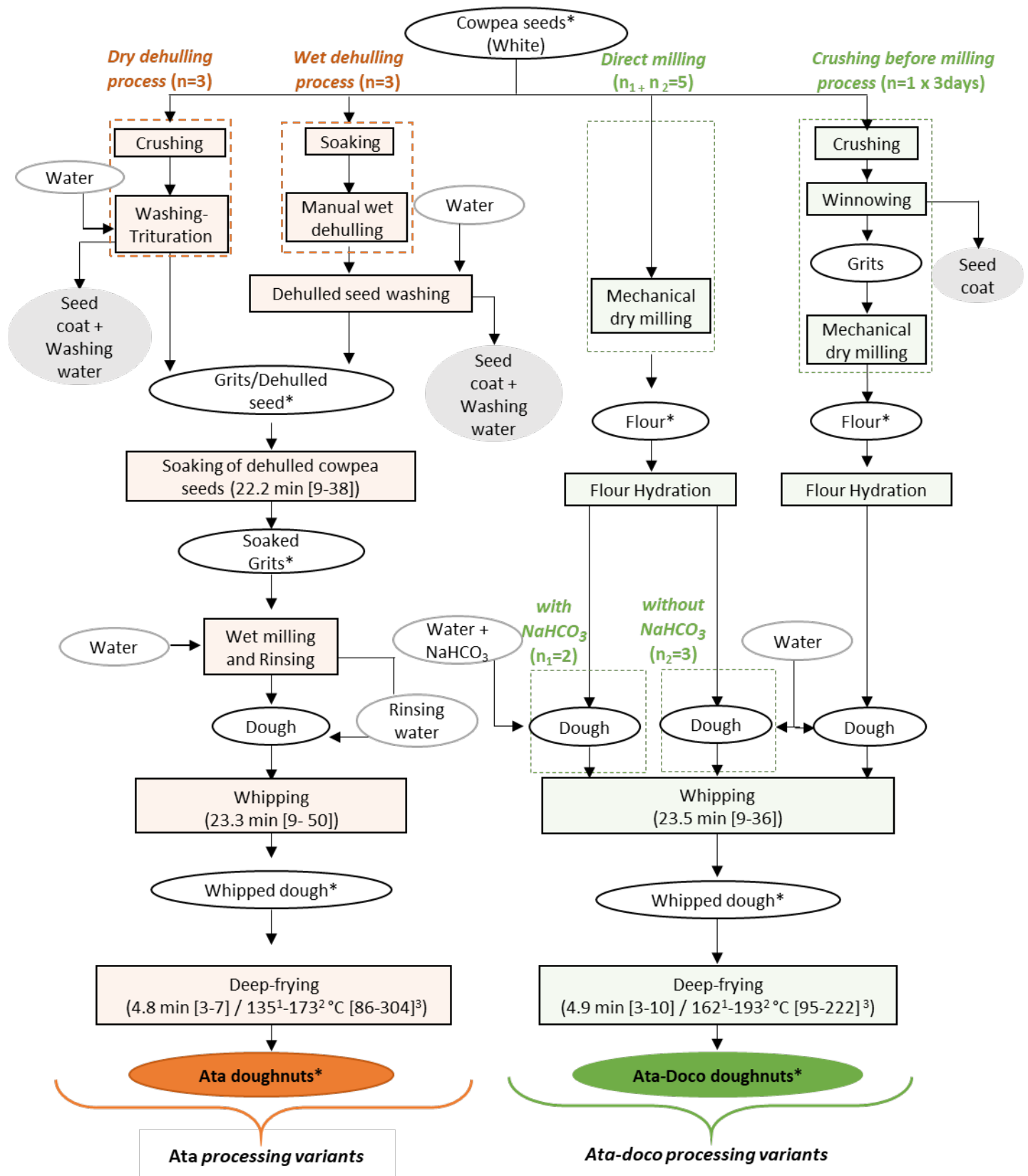


Figure 1: Diagram showing the different steps of cowpea seed processing into ata and ata-doco. <sup>1</sup>: Mean initial temperature (T<sub>0</sub>); <sup>2</sup>: Mean final temperature (T<sub>F</sub>); <sup>3</sup>: [Min value T<sub>0</sub> - Max value T<sub>F</sub>]; \* indicates sampling points; 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).

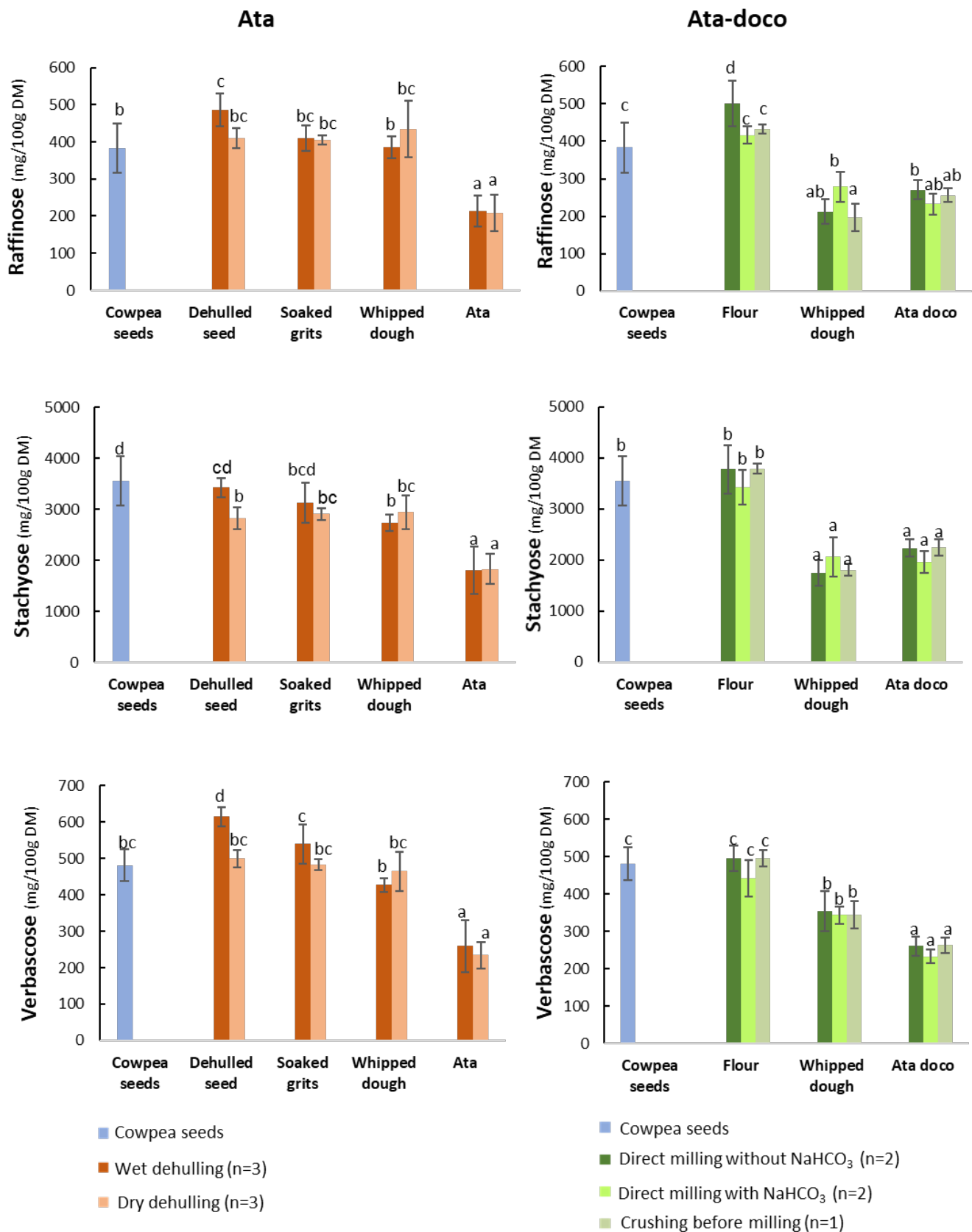


Figure 2: **Raffinose, stachyose and verbascose content (on a dry matter basis) during ata and ata-doco) processing.** n: number of producers, Different superscript letters correspond to significant difference between samples ( $p < 0.05$ , Newman-Keuls test was used for post-hoc comparison).

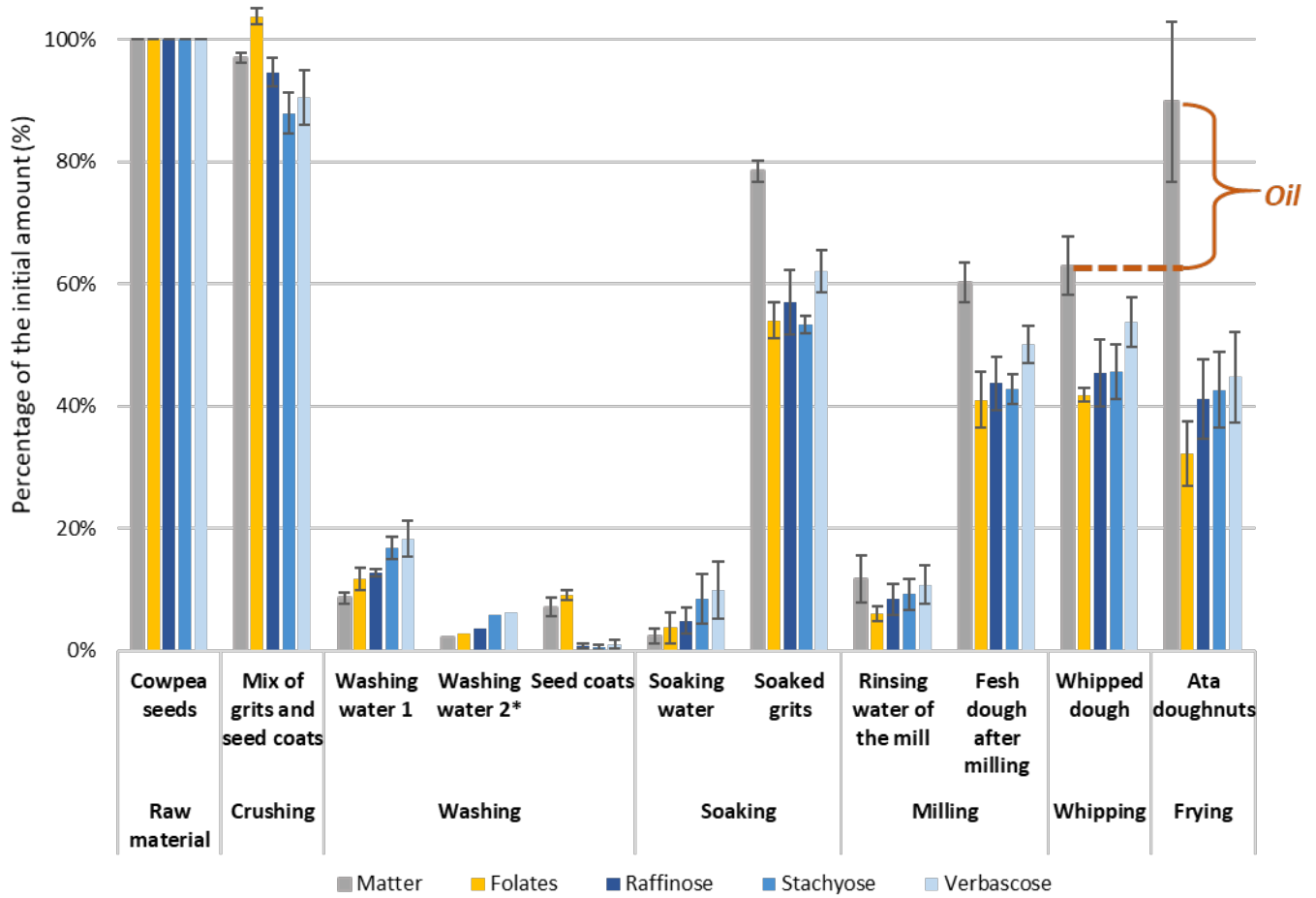


Figure 3: **Dry matter, folate and alpha-galactoside distribution in the different production fractions during the traditional processing of ata** (calculated using the dry weight of each fraction and component content; data are the mean values for the samples collected from three producers). Oil: proportion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

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

Products	Process	Dry matter	Protein	Fat	Available carbohydrate**	Ash	Dietary fibres			Energy
		(g/100g)	(g/100g DM)				(g/100g DM)			(Kcal/100g)
							IDF	SDF	TDF	
<b>Cowpea seeds</b>		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
<b>Ata</b>	Wet dehulling (n=3)	58 ± 1.0 <sup>a</sup>	14.0 ± 1.4 <sup>a</sup>	42.7 ± 2.0 <sup>b</sup>	31 ± 3.4 <sup>ab</sup>	2.6 ± 0.9 <sup>a</sup>	8.4 ± 0.4 <sup>b</sup>	1.2 ± 0.3 <sup>a</sup>	9.6 ± 0.4 <sup>b</sup>	337 ± 12 <sup>b</sup>
	Mechanical dry dehulling (n=3)	61 ± 9.7 <sup>a</sup>	14.2 ± 3.2 <sup>a</sup>	52.9 ± 13 <sup>c</sup>	24 ± 16 <sup>a</sup>	2.9 ± 0.2 <sup>a</sup>	5.2 ± 0.6 <sup>a</sup>	1.3 ± 0.4 <sup>a</sup>	6.5 ± 0.3 <sup>a</sup>	388 ± 36 <sup>c</sup>
<b>Ata-doco</b>	Direct milling (+) NaHCO <sub>3</sub> (n=2)	54 ± 3.6 <sup>a</sup>	17.1 ± 1.2 <sup>b</sup>	32.4 ± 2.4 <sup>a</sup>	36 ± 3.1 <sup>b</sup>	3.4 ± 0.3 <sup>a</sup>	8.6 ± 1.1 <sup>b</sup>	2.2 ± 0.3 <sup>b</sup>	10.8 ± 1.5 <sup>b</sup>	285 ± 17 <sup>a</sup>
	Direct milling (-) NaHCO <sub>3</sub> (n=3)	53 ± 3.5 <sup>a</sup>	17.9 ± 1.0 <sup>b</sup>	25.6 ± 5.5 <sup>a</sup>	40 ± 6.6 <sup>b</sup>	5.4 ± 1.4 <sup>b</sup>	9.0 ± 0.9 <sup>b</sup>	2.3 ± 0.4 <sup>b</sup>	11.3 ± 0.8 <sup>b</sup>	254 ± 8 <sup>a</sup>
	Crushing before milling (n=1) *	58 ± 4.4 <sup>a</sup>	15.8 ± 0.8 <sup>ab</sup>	27.1 ± 2.5 <sup>a</sup>	41 ± 2.2 <sup>b</sup>	6.0 ± 1.0 <sup>b</sup>	8.3 ± 0.6 <sup>b</sup>	1.7 ± 0.6 <sup>ab</sup>	10.0 ± 1.2 <sup>b</sup>	283 ± 30 <sup>a</sup>
<b>Ata vs. Ata-doco</b>	<i>P-value</i>	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).

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

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

**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 ± standard deviation (samples were collected from the indicated number of producers). \*: Samples were collected from this producer on three different days.



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

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

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.